





Bovine tuberculosis

Tuberculosis in cattle

Improving surveillance	10
Improving management of high risk herds	16
Supporting studies	24

The role of wildlife in bovine TB

Improved understanding of ecology and TB epidemiology	30
Infection control strategies	38
Supporting studies	51

Contributing to national TB policy

The national programme	54
Quality control	55
Public health	56
North-South linkages	57

National maps

Density of TB incidence	58
APT per DED	60

Improving surveillance

Field-based surveillance

Multiplex immunoassay for serological diagnosis of *Mycobacterium bovis* infection in cattle

Whelan, C.¹, Shuralev, E.¹, O'Keeffe, G.¹, Hyland, P.¹, Kwok, H.F.², Snoddy, P.², O'Brien, A.¹, Connolly, M.¹, Quinn, P.¹, Groll, M.³, Watterson, T.³, Call, S.³, Kenny, K.⁴, Duignan, A.^{5, 8}, Hamilton, M.J.⁶, Buddle, B.M.⁷, Johnston, J.A.², Davis, W.C.⁶, Olwill, S.A.², Clarke, J.¹

¹ Enfer Scientific, ² Fusion Antibodies Ltd., Northern Ireland, ³ Quansys Biosciences, Utah, USA, ⁴ Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food, ⁵ Department of Agriculture, Fisheries and Food, ⁶ Washington State University, USA, ⁷ AgResearch, Hopkirk Research Institute, Palmerston North, New Zealand, ⁸ UCD CVERA

Clinical and Vaccine Immunology 15, 1834-1838 (2008)

Efforts to develop a better diagnostic assay for bovine tuberculosis have shown that the sensitivity and specificity of an assay can be improved by the use of two or more antigens. As reported here, we developed a multiplex chemiluminescent immunoassay that can simultaneously detect antibody activity to 25 antigens in a single well in a 96-well plate array format. The chemiluminescent signal is captured with a digital imaging system and analyzed with a macro program that tracks each serum for its pattern of antibody activity for *Mycobacterium bovis* antigens. The comparison of sera from 522 infected and 1,489 uninfected animals showed that a sensitivity of 93.1% and a specificity of 98.4% can be achieved with a combination of antigens. The assay system is rapid and can be automated for use in a centralized laboratory.

Reproduced with permission from the American Society for Microbiology.

Using latent class analysis to estimate the test characteristics of the interferon- γ test, the single intradermal comparative tuberculin test (SICTT) and a multiplex immunoassay under Irish conditions

Clegg, T.A.¹, Duignan, A.^{1, 2}, Whelan, C.³, Gormley, E.⁴, Good, M.², Clarke, J.³, Toft, N.⁵, More, S.J.¹

¹ UCD CVERA, ² Department of Agriculture, Fisheries and Food, ³ Enfer Scientific, ⁴ UCD TB Diagnostics and Immunology Research Centre, ⁵ University of Copenhagen, Copenhagen, Denmark

Considerable effort has been devoted to improving existing diagnostic tests for bovine tuberculosis (single intradermal comparative tuberculin test [SICTT] and γ -interferon assay [γ IFN]) and to develop new tests. Previously, the diagnostic characteristics (sensitivity, specificity) have been estimated in populations with defined infection status. However, these approaches can be problematic as there may be few herds in Ireland where freedom from infection is guaranteed. We used latent class models to estimate the diagnostic characteristics of existing (SICTT and γ IFN) and new (multiplex immunoassay [Enferplex-TB]) diagnostic tests under Irish field conditions where true disease status was unknown. The study population consisted of herds recruited in areas with no known TB problems (2,197 animals) and herds experiencing a confirmed TB breakdown (2,740 animals). A Bayesian model was developed, allowing for dependence between SICTT and γ IFN, while assuming independence from the Enferplex-TB test. Different test interpretations were used for the analysis: SICTT (standard and severe interpretation), γ IFN (a single interpretation), and a range of interpretations for the Enferplex-TB (level-1 [high sensitivity interpretation] to level-5 [high specificity interpretation]). The sensitivity and specificity (95% posterior credibility intervals; 95% PCI) of SICTT[standard] relative to Enferplex-TB[level-1] and γ IFN were 52.9-60.8% and 99.2-99.8%, respectively. Equivalent estimates for γ IFN relative to Enferplex-TB[level-1] and SICTT were 63.1-70.1% and 86.8-89.4%, respectively. Sensitivity of

Enferplex-TB[level-1] (95% PCI: 64.8-71.9%) was superior to the SICTT[standard], and specificity of the Enferplex-TB[level-5] was superior to γ IFN (95% PCI: 99.6-100.0%). These results provide robust measures of sensitivity and specificity under field conditions in Ireland and suggest that the Enferplex-TB test has the potential to improve on current diagnostics for TB infection in cattle.

Further test characterisation (comparison of herd-level classification using SICTT and Enfer)

Good, M.¹, Clegg, T.A.²

¹ Department of Agriculture, Fisheries and Food, ² UCD CVERA

For the purpose of this study DVOs submitted blood samples for analysis from herds undergoing high risk or consequential test type tests (i.e. not round tests) and which represent the whole of Ireland. Samples were also 'captured' from the Brucellosis Laboratory for herds undergoing routine round tests. In total some 100,000 samples were analysed by Enfer Scientific in the latter months of 2009. The principal research questions to be addressed by this study are as follows:

- Is there agreement in the herd-level interpretation of the SICTT and Enferplex tests?
- If not, what is the confirmation rate among SICTT herds where no animals were Enferplex positive?
- What is the future outcome of herds/animals that were Enferplex positive but SICTT negative?

Meta-analysis of diagnostic tests and modelling to identify appropriate testing strategies to reduce *Mycobacterium bovis* infection in cattle

Downs, S.¹, Parry, J.¹, Abernethy, D.², Broughan, J.¹, Cameron, A.³, Clifton-Hadley, R.¹, Cook, A.¹, Cox, D.⁴, de la Rua-Domenech, R.⁵, Goodchild, T.¹, Greiner, M.⁶, Gunn, J.¹, More, S.J.⁷, Nunez-Garcia, J.¹, Sharp, M.¹, Rhodes, S.¹, Rolfe, S.⁸, Upton, P.¹, Vordermeier, M.¹, Watson, E.¹, Welsh, M.⁹, Whelan, A.¹, Woolliams, J.¹⁰

¹ Veterinary Laboratories Agency, Weybridge, Surrey, England, ² Department of Agriculture and Rural Development, Belfast, Northern Ireland, ³ AusVet Animal Health Services, Cuiseaux, France, ⁴ Nuffield College, University of Oxford, Oxford, England, ⁵ Department of Environment, Food and Rural Affairs, United Kingdom, ⁶ Federal Institute for Risk Assessment (BfR), Berlin, Germany, ⁷ UCD CVERA, ⁸ Welsh Assembly Government, Cardiff, Wales,

⁹ Agri-Food & Biosciences Institute (AFBI), Belfast, Northern Ireland, ¹⁰ Roslin Institute of the University of Edinburgh, Scotland

In this collaborative project, a comprehensive systematic literature review has been undertaken of studies that have measured the performance of tests for diagnosing bovine tuberculosis in cattle. Estimates of test performance and the characteristics of the studies and populations in which the studies were conducted have been extracted from the literature, following a standardised procedure. The results will be pooled and a statistical meta-analysis conducted to obtain estimates with distributions for the sensitivities and specificities of the tests. The second part of the project is the development of a model to explore the improved use of the tests in bovine tuberculosis surveillance and control. The model estimates the probability that a herd with specified characteristics and surveillance history is free from infection. The model will be used to evaluate a range of potential future surveillance options, based on sensitivity, specificity, the probability of introduction of infection into a herd and the time required to achieve *M. bovis* infection free status.

The relative effectiveness of, and reporting accuracy among, testers during field surveillance for bovine tuberculosis in Ireland

Clegg, T.A.¹, Good, M.², Duignan, A.^{1, 2}, More, S.J.¹

¹ UCD CVERA, ² Department of Agriculture, Fisheries and Food

In Ireland, cattle are tested annually, using the single intradermal comparative tuberculin test (SICTT). Quality control (QC) is an important part of the national programme, and new methods are progressively being introduced. Field surveillance using the SICTT is potentially problematic, noting its reliance on a range of factors, including the skills and experience of the tester. The objective of the current study was to quantify the relative effectiveness of, and reporting accuracy among, testers during field surveillance for tuberculosis in Ireland. All testers who carried out at least one annual herd test in 2006 were included in the study. Relative testing effectiveness was assessed by comparing the number of observed and expected herd restrictions per tester. The latter was predicted, using a logistic regression model and testers were ranked, based on OEDIFF. Reporting accuracy was based on the bovine measurements in SICTT negative animals. In total, 983 testers were enrolled in the study, including data from 92,402 herds, including 1,839 (1.99%) with at least one reactor at the eligible test. OEDIFF varied from -7.5 to 18.2; for 43% of testers, OEDIFF was between -1.0 and 1.0. There was evidence of reduced testing effectiveness among a small number of testers. Reporting accuracy was low suggesting under-reporting of bovine measurements. This study provides objective data on the relative performance of testers during field surveillance in Ireland. Output from these models will be used as part of ongoing QC activities in the national programme.

A review of quality control in the national bovine tuberculosis eradication programme in Ireland

Duignan, A.^{1, 2}, Good, M.¹, More, S.J.²

¹ Department of Agriculture, Fisheries and Food, ² UCD CVERA

Quality control (QC) is a recognised process in the delivery of quality products or services. The application of QC in veterinary laboratories has been described in some detail; as yet, however, little has been written about QC in other aspects of national animal disease control programmes. This paper presents a review of QC in the national bovine tuberculosis (BTB) eradication programme in Ireland, with particular emphasis on QC processes for Private Veterinary Practitioners (PVPs) who carry out much of the field surveillance using the Single Intradermal Comparative Tuberculin Test (SICTT). The Irish BTB eradication programme operates under national legislation and at the same time fulfils the requirements of the EU trade Directive 64/432. The programme includes annual SICTT screening of all herds, prompt removal of test reactors and further consequential retesting of herds. Continuous evaluation of all relevant activities is essential to deliver an effective national programme and to reassure all stakeholders including taxpayers, producers and export markets that the highest possible standards are attained. A broad range of programme elements subjected to QC, are described, including personnel, training, equipment, tuberculins and laboratory. Each element is relevant to activities within both field and abattoir surveillance. Particular attention is paid to field surveillance (specifically, PVP performance), following the recent introduction of the national Animal Health Computer System (AHCS). Specialist performance reports have been produced, focusing on measures relating to administrative functions and disease control. The measures were chosen as objective and measurable. They are perceived as the best available and the process is constantly evolving as part of a work in progress. While attention has been directed to the issue of standards of SICTT delivery by PVPs, little has been documented in published literature on the subject of Quality Control and Quality Assurance in disease eradication programmes. This paper seeks to fill that gap.

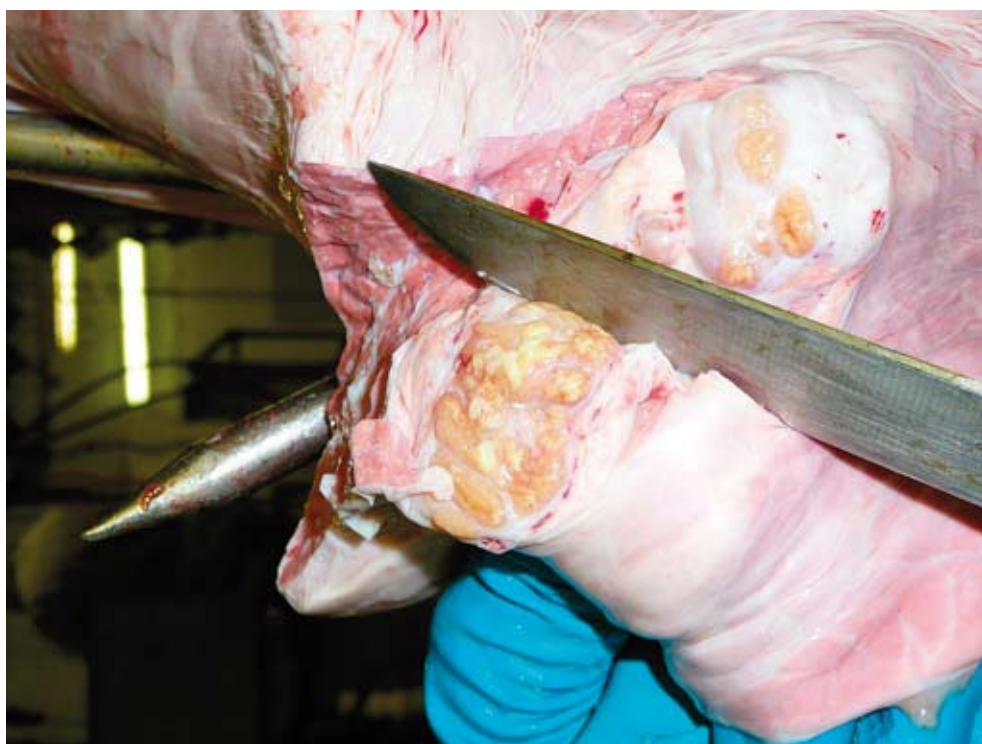
Factory surveillance

Quantification of the relative efficiency of factory surveillance in the disclosure of tuberculosis lesions in attested Irish cattle, 2005-2007

Olea-Popelka, F.J.¹, Freeman, Z.¹, White, P.^{2, 3}, Costello, E.⁴, O’Keeffe, J.^{2, 3}, Frankena, K.⁵, Martin, S.W.⁶, More, S.J.³

¹ Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Colorado, USA, ² Department of Agriculture, Fisheries and Food, ³ UCD CVERA, ⁴ Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food, ⁵ Wageningen Institute of Animal Sciences, Wageningen University, The Netherlands, ⁶ Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada

In Ireland, as part of the ongoing Bovine Tuberculosis (BTB) eradication scheme, every animal is examined at slaughter for its fitness for human consumption. Between 1982 and 1999, the annual risk of BTB lesion detection among animals from herds considered to be free of BTB was 0.12% (on average 1,638 animals per year) (Byrne, 2000). The aim of this study was to determine the relative efficiency of factories in detecting, submitting and subsequently confirming BTB lesion as caused by *Mycobacterium bovis* among attested cattle (considered free of BTB) between years 2005-2007. Factories were ranked according their submission and confirmation risk, adjusting for the risk profile of the animals slaughtered, including potential confounding factors such as age, gender, whether they were homebred or purchased, the test history of their herd, the prevalence of BTB in the herd geographical area and the season of slaughter. Approximately, 4.9 million cattle were slaughtered in 37 Irish export-licensed factories in these three years. Complete data were available for 3,344,057 animals from 89,870 attested herds in 2830 District Electoral Divisions. Samples from 11,530 attested animals with suspected BTB lesions were submitted for laboratory examination, 7,900 (68.5%) were positive, 3,238 were negative and 392 were inconclusive. Samples from 8,178 animals with complete information regarding potential confounding factors with suspected BTB lesions were submitted for laboratory examination, and from these 5,456 (66.7%) were positive, 2453 were negative and 269 were inconclusive. The average unadjusted submission risk for all the factories was 25 per 10,000, ranging from 0 to 52 per 10,000. The unadjusted confirmation risk varied between 30.3% and 91.3%.



Incised lung during factory surveillance. Photograph by A. Duignan.

Supporting work

Antigen stimulation of peripheral blood mononuclear cells from *Mycobacterium bovis* infected cattle yields evidence for a novel gene expression programMeade, K.G.¹, Gormley, E.², O'Farrelly, C.¹, Park, S.D.³, Costello, E.⁴, Keane, J.⁵, Zhao, Y.⁶, MacHugh, D.E.^{3, 7}¹ School of Biochemistry and Immunology, Trinity College Dublin, ² UCD TB Diagnostics and Immunology Research Centre, ³ UCD School of Agriculture, Food Science and Veterinary Medicine, ⁴ Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food, ⁵ School of Medicine, Trinity College Dublin, ⁶ Computational and Systems Biology Group, Biometric Research Branch, National Cancer Institute, Maryland, USA, ⁷ UCD Conway Institute of Biomolecular and Biomedical Research

BMC Genomics 9, 447 (2008)

Bovine tuberculosis (BTB) caused by *Mycobacterium bovis* continues to cause substantial losses to global agriculture and has significant repercussions for human health. The advent of high throughput genomics has facilitated large scale gene expression analyses that present a novel opportunity for revealing the molecular mechanisms underlying mycobacterial infection. Using this approach, we have previously shown that innate immune genes in peripheral blood mononuclear cells (PBMC) from BTB-infected animals are repressed in vivo in the absence of exogenous antigen stimulation. In the present study, we hypothesized that the PBMC from BTB-infected cattle would display a distinct gene expression program resulting from exposure to *M. bovis*. A functional genomics approach was used to examine the immune response of BTB-infected (n = 6) and healthy control (n = 6) cattle to stimulation with bovine tuberculin (purified protein derivative - PPD-b) in vitro. PBMC were harvested before, and at 3 h and 12 h post in vitro stimulation with bovine tuberculin. Gene expression changes were catalogued within each group using a reference hybridization design and a targeted immunospecific cDNA microarray platform (BOTL-5) with 4,800 spot features representing 1,391 genes. 250 gene spot features were significantly differentially expressed in BTB-infected animals at 3 h post-stimulation contrasting with only 88 gene spot features in the non-infected control animals ($P < \text{or} = 0.05$). At 12 h post-stimulation, 56 and 80 gene spot features were differentially expressed in both groups respectively. The results provided evidence of a proinflammatory gene expression profile in PBMC from BTB-infected animals in response to antigen stimulation. Furthermore, a common panel of eighteen genes, including transcription factors were significantly expressed in opposite directions in both groups. Real-time quantitative reverse transcription PCR (qRT-PCR) demonstrated that many innate immune genes, including components of the TLR pathway and cytokines were differentially expressed in BTB-infected (n = 8) versus control animals (n = 8) after stimulation with bovine tuberculin. The PBMC from BTB-infected animals exhibit different transcriptional profiles compared with PBMC from healthy control animals in response to *M. bovis* antigen stimulation, providing evidence of a novel gene expression program due to *M. bovis* exposure.

Printed with permission from BioMed Central and authors.

Gene expression profiling of the host response to *Mycobacterium bovis* infection in cattle

MacHugh, D.E.^{1, 2}, Gormley, E.³, Park, S.D.¹, Browne, J.A.¹, Taraktoglou, M.¹, O'Farrelly, C.⁴, Meade, K.G.⁴

¹ UCD School of Agriculture, Food Science and Veterinary Medicine, ² UCD Conway Institute of Biomolecular and Biomedical Research, ³ UCD TB Diagnostics and Immunology Research Centre, ⁴ Comparative Immunology Group, School of Biochemistry and Immunology, Trinity College Dublin

Transboundary and Emerging Disease 56, 204-214 (2009)

Bovine tuberculosis (BTB), caused by *Mycobacterium bovis*, continues to pose a threat to livestock worldwide and, as a zoonotic infection, also has serious implications for human health. The implementation of comprehensive surveillance programmes to detect BTB has been successful in reducing the incidence of infection in many countries, yet BTB has remained recalcitrant to eradication in several EU states, particularly in Ireland and the UK. There are well-recognized limitations in the use of the current diagnostics to detect all infected animals and this has led to renewed efforts to uncover novel diagnostic biomarkers that may serve to enhance the performance of the tests. Studies of single immunological parameters have so far been unable to unlock the complexities of the immune response to mycobacterial infection. However, the development of high-throughput methods including pan-genomic gene expression technologies such as DNA microarrays has facilitated the simultaneous identification and analysis of thousands of genes and their interactions during the immune response. In addition, the application of these new genomic technologies to BTB has identified pathogen-associated immune response signatures of host infection. The objective of these investigations is to understand the changing profile of immune responses throughout the course of infection and to identify biomarkers for sensitive diagnosis, particularly during the early stages of infection. Transcriptional profiling via microarray and more recently via next-generation sequencing technologies may lead to the development of specific and sensitive diagnostics for *M. bovis* infection and will enhance the prospect of eradication of tuberculosis from cattle populations.

Printed with permission from John Wiley & Sons, Inc.

The original article can be found at <http://www3.interscience.wiley.com/journal/122404541/abstract>



Photograph courtesy of Bord Bia.

Improving management of high risk herds

TB epidemiology

Risk of bovine tuberculosis (BTB) for cattle sold out from attested herds during year 2005 in Ireland

Berrian, A.M.¹, O'Keeffe, J.^{2, 3}, White, P.^{2, 3}, Olea-Popelka, F.⁴

¹ Colorado School of Public Health, Colorado, USA, ² Department of Agriculture, Fisheries and Food, ³ UCD CVERA, ⁴ Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Colorado, USA

Bovine tuberculosis (BTB) is an infectious contagious disease caused by a bacterium *Mycobacterium bovis*. The significance of BTB lies in trade implications and its zoonotic potential. Although industrialized countries have significantly reduced the prevalence of *M. bovis* infection in both humans and animals, BTB persists in many developed countries, including the Republic of Ireland. A retrospective cohort study was conducted to determine the risk of BTB among animals sold out from attested (BTB “free”) herds during the year 2005. Herds from which the animals were sold out were classified as “exposed” and “non-exposed” to BTB according to their BTB history during the year 2005. The study sample was comprised of 338,960 animals, from which 124,360 were sold out from “exposed” herds and 214,600 animals were sold out from “non-exposed” herds. After the selling date during the year 2005, animals were followed until the end of the year 2007. All animals reacting positive to the Single Intradermal Comparative Tuberculin Test (SICTT) as well as all animals that disclosed a BTB lesion at slaughter that subsequently was confirmed as *M. bovis* positive at the laboratory were classified as BTB positive. The overall risk of BTB during the study period after the animals were sold out was 0.69%. Logistic regression analysis indicated that the odds of being found BTB positive was greater for animals sold out from “exposed” herds compared to animals sold out from “non-exposed” herds (OR=1.94, 95% CI = 1.79-2.11, $p = < 0.0001$). Other risk factors, such as age and gender, were also significantly associated with the risk of BTB at the animal level in Ireland. Due to the contagious nature of BTB, the identification and quantification of risk factors associated with the development of the disease is essential to its control at the animal level. These are preliminary findings from a study to be completed during the year 2010. This study will provide quantitative epidemiological information at the animal level that can be used to further assess Ireland's progress towards BTB eradication.

Tuberculosis in a dairy farm in Ireland: a case study

Meaney, L.¹, Kenny, K.², Johnson, A.³, Good, M.¹

¹ Department of Agriculture, Fisheries and Food, ² Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food,

³ Limerick Regional Veterinary Laboratory, Department of Agriculture, Fisheries and Food

In a number of countries, bovine tuberculosis (BTB; due to infection with *Mycobacterium bovis*) is a significant animal health problem. In Ireland, BTB is a problem in the national cattle herd, and is endemic in badgers (*Meles meles*). However, few case reports from Ireland are available. The aim of this paper is to describe an outbreak of bovine tuberculosis in a dairy herd on a farm in Ireland, where 3% of the herd was SICTT-positive at initial detection but where infection was eventually evident in all groups within the herd. The epidemiological investigation was conducted in an effort to determine the origin of the outbreak and within-herd spread, including consideration of the possibility of spread to and from people on the holding.

How important is introduced infection?

Potential infection-control benefit for Ireland from pre-movement testing of cattle for tuberculosis

Clegg, T.A.¹, More, S.J.¹, Higgins, I.¹, Good, M.², Blake, M.², Williams, D.H.³

¹ UCD CVERA, ² Department of Agriculture, Fisheries and Food, ³ UCD School of Mathematical Sciences

Preventive Veterinary Medicine 84, 94-111 (2008)

Pre-movement testing for bovine tuberculosis (BTB) was compulsory in Ireland until 1996. We determined the proportion of herd restrictions (losing BTB-free status) attributable to the recent introduction of an infected bovid; described events between restoration of BTB-free status (de-restriction) and the next herd-level test for BTB; estimated the proportion of undetected infected cattle present at de-restriction; identified high-risk movements between herds (movements most likely to involve infected cattle); and determined the potential yield of infected cattle discovered (or herds that would not lose their BTB-free status) by pre-movement testing, relative to the numbers of cattle and herds tested. We used national data for all 6252 herds with a new BTB restriction in the 12 months from 1 April 2003 and 3947 herds declared BTB free in the 12 months from 1 October 2001. We identified higher-risk animals from our logistic generalized estimating-equation models. We attributed 6–7% of current herd restrictions to the recent introduction of an infected animal. There were considerable changes to herd structure between de-restriction and the next full-herd test, and infection was detected in 10% of herds at the first assessment (full-herd test or abattoir surveillance) following de-restriction. Following movement from a de-restricted herd, the odds of an animal being positive at the next test increased with increasing time in the source herd prior to movement, increasing time between de-restriction and the next full-herd test and increasing severity of the source herd restriction. The odds decreased with increasing size of the source herd. We estimated that 15.9 destination-herd restrictions per year could be prevented for every 10,000 cattle tested pre-movement and that 3.3 destination-herd restrictions per year could be prevented for every 100 source herds tested pre-movement. The yield per pre-movement test can be increased by focusing on high-risk movements; however, this would result in a substantial decrease in the total number of potential restrictions identified.

This article was published in Preventive Veterinary Medicine, 84, Clegg, T.A., More, S.J., Higgins, I., Good, M., Blake, M., Williams, D.H., Potential infection-control benefit for Ireland from pre-movement testing of cattle for tuberculosis, 94-111, Copyright Elsevier B.V. 2008.

Predictors of the first between-herd animal movement for cattle born in 2002 in Ireland - the implications for control/eradication of bovine tuberculosis

White, P.^{1, 2}, Frankena, K.³, de Jong, M.³, O'Keeffe, J.^{1, 2}, Martin, W.⁴

¹ Department of Agriculture, Fisheries and Food, ² UCD CVERA, ³ Wageningen Institute of Animal Sciences, Wageningen University, The Netherlands,

⁴ Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada

The movement of cattle between herds may constitute a risk for the transmission of bovine tuberculosis (BTB) and other disease transmission. The objectives of this study were to identify and quantify the risk factors associated with the first between-herd movement of animals (denoted as risk move) as an aide to control BTB in Ireland. A random sample of 1 percent of Irish calf births registered for 2002 (20,182 animals) was selected. Descriptive and survival analysis on movement over the period 2002-2005 was performed. Over the 4-year study period, 12,119 (60%) of animals experienced a risk move. Among those that moved, 57% did so within the first 12 months of age. For animals in dairy herds, an early peak in risk move events was observed within the first 12 weeks of age; whereas in animals from suckler herds, a later risk move peak was observed between 21 and 36 weeks of age. The survival models identified a number of risk factors:

two that appeared most important in predicting a risk move were gender and enterprise type. Males had a hazard ratio of 2.6 times that of females. The hazards for enterprise type, varied over time, thus a time-varying covariate (ent_type x ln(time)) was included in the Cox model. At 7 days of age, females in suckler herds were at 0.14 times the hazard of females in dairy herds for risk move, and over time, the hazards converged, equalised by day 140, and then diverged, so that by 4 years of age, females in suckler herds were at 4.64 times the hazard of females in dairy herds. Herds with a history of selling animals in previous years maintained that record during the study period with increased hazard of risk move. Enterprise type interacted with gender so that relative to females, males from dairy herds were at greater hazard of risk move than males from suckler herds. Hazard of risk move was also a function of ln(herd area), so that each doubling of farm area was accompanied by a 30.6% decrease in the hazards. When the BTB outcome for the selected animals was examined for the period 2002-2005, cattle that had a risk move were less likely to either fail a tuberculin test, or show confirmed factory lesion(s) at slaughter than those without a risk move (0.52% and 0.97% respectively).

The BTB risk associated with purchasing cattle during a herd restriction

Higgins, I.¹, Clegg, T.A.¹, Valy, L.², More, S.J.¹

¹ UCD CVERA, ² Ecole Nationale Vétérinaire de Toulouse, Toulouse, France

In Ireland, herds are restricted from trading for a (defined) period after TB has been detected. In some of these cases, the purchasing of animals into the restricted herd is allowed under permit to replace animals (production units) that were lost as a result of TB or to enable routine management practices (such as winter feeding of calves) to continue 'as normal' (in spite of the TB restrictions imposed). Concern has been raised as to whether this practice extends the restriction period.

Therefore, the objectives of this study are three-fold:

- To determine whether the purchasing of animals into a restricted herd is associated with increased TB risk
- To provide an overview of events associated with each purchasing episode and to clarify the infection status of animals that are introduced during restrictions
- If there is evidence of an increased TB risk, to identify the practices relating to the purchasing of animals that are the most risky and to determine whether the increased risk is associated with the source herd or the purchasing herd under restriction

To date, data has been extracted to identify the eligible population that were not restricted at the start of 2006, but experienced a TB episode during that year. Herds with unusual patterns of trading were excluded. Preliminary data analyses have been performed and further work is necessary to determine:

- Which practices relating to purchasing of animals during a restriction are the most risky, and
- Whether there is an increased risk associated with the source herd, the purchasing herd under restriction or both.

Two outcomes of interest have been identified, including duration of restriction (based on start and end dates) and time from de-restriction to subsequent breakdown or end of study [31DEC08], whichever occurred first.

Disentangling the relative importance of residual infection, contiguous spread and locality

Risk factors for disclosure of additional tuberculous cattle in attested-clear herds that had one animal with a confirmed lesion of tuberculosis at slaughter during 2003 in Ireland

Olea-Popelka, F.P.^{1, 2}, Costello, E.³, White, P.^{4, 5}, McGrath, G.⁵, Collins, J.D.⁵, O’Keeffe, J.^{4, 5}, Kelton, D.F.¹, Berke, O.¹, More, S.⁵, Martin, S.W.¹

¹ Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada, ² Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Colorado, USA, ³ Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food, ⁴ Department of Agriculture, Fisheries and Food, ⁵ UCD CVERA

Preventive Veterinary Medicine 85, 81-91 (2008)

All the Irish cattle herds considered “clear” of bovine tuberculosis (BTB) having a single animal with a tuberculous lesion at slaughter during 2003 were identified. We performed a descriptive and logistic regression analysis to investigate whether selected risk factors had an association with the result of the herd test immediately after the tuberculous lesion was found (“Factory Lesion Test”, FLT). At the FLT, only 19.7% ($n = 338$) of these 1713 herds had 1 or more standard reactors. The lesioned animal was home-bred in 46% of the “source” herds; these herds had an increased risk (23.4%) of having at least 1 standard reactor animal relative to herds with a purchased-lesioned animal (16.6%) (RR = 1.41). Our logistic models identified a number of important risk factors; two that appeared most important in predicting the FLT outcome were the time spent (residency) by the lesioned animal in the “source” herd, and the presence, or not, of the lesioned animal in a previous BTB episode in either the “source” herd, or the seller’s herd in the case the lesioned animal was purchased. Our models fit the data well based on the Hosmer–Lemeshow test, however their sensitivity and specificity were very low (57% and 61% respectively). Surveillance of the cattle population for BTB using lesions found at slaughter is an essential component of an overall control program. Nonetheless, due to the poor predictability of the variables we measured, complete herd investigations are needed to help explain the FLT outcome of a herd.

This article was published in Preventive Veterinary Medicine, 85, Olea-Popelka, F.P., Costello, E., White, P., McGrath, G., Collins, J.D., O’Keeffe, J., Kelton, D.F., Berke, O., More, S., Martin, S.W., Risk factors for disclosure of additional tuberculous cattle in attested-clear herds that had one animal with a confirmed lesion of tuberculosis at slaughter during 2003 in Ireland, 81-91, Copyright Elsevier B.V. 2008.

Estimation of the between-herd reproduction ratio for contiguous spread of bovine tuberculosis

White, P.^{1, 2}, Frankena, K.³, O’Keeffe, J.^{1, 2}, More, S.J.², de Jong, M.³, Martin, S.W.⁴

¹ Department of Agriculture, Fisheries and Food, ² UCD CVERA, ³ Wageningen Institute of Animal Sciences, Wageningen University, The Netherlands, ⁴ Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada

The aim of any eradication programme is to bring the reproductive ratio for the disease below 1. Tuberculin testing data is available from the Animal Health Computer System (AHCS) database for years 1989 to 2007. Together with contiguous data from the Land Parcel Information System, these data will be used for the analysis of possible transmission pathways for bovine tuberculosis between neighbouring herds following initial infection. After back-calculation to determine the sequence of infection between herds, the likelihood of between-herd transmission will be evaluated and an overall reproduction ratio calculated for contiguous spread.

An evaluation of the Irish single reactor breakdown protocol for 2005 to 2008 and its use as a monitor of tuberculin test performance

Good, M.¹, Duignan, A.^{1, 2}

¹ Department of Agriculture, Fisheries and Food, ² UCD CVERA

Under the Irish Bovine Tuberculosis (BTB) Eradication Programme, all herds are subjected to at least one test per annum. The Single Intra-dermal Comparative Tuberculin Test (SICTT) is used in Ireland for the detection of cattle infected with *Mycobacterium bovis*. There have been concerns regarding the specificity of the SICTT, notably by farmers, and particularly in herds where the detection of a single positive animal in the absence of an obvious source of (BTB) infection could be perceived as a “false” positive. To address this issue, the so-called ‘Singleton Protocol’ was established as part of the Irish BTB eradication programme. This protocol allows for the early restoration of free trading status to herds where a single positive animal was detected and where the herd was not confirmed as infected with *M. bovis* by epidemiological investigation, by *post mortem*, by laboratory examination, or by further test. This paper presents:

- data from the 2005 to 2008 BTB programmes on the number of herds which were assessed and which qualified for inclusion under the ‘Singleton Protocol’, and
- the outcome for qualifying herds up to and including having status restored early as a consequence of inclusion in that programme.

The outcome of this protocol reaffirms the reliability of the SICTT at current levels of infection. However, as overall infection levels of BTB fall, it is advocated that the ‘Singleton Protocol’ be continued as a monitor of herds in which a single positive animal is disclosed, to assess progress towards BTB eradication in Ireland.

Future risk of *Mycobacterium bovis* infection following an inconclusive diagnosis

Clegg, T.A.¹, Good, M.², Duignan, A.^{1, 2}, Doyle, R.², More, S.J.¹

¹ UCD CVERA, ² Department of Agriculture, Fisheries and Food

A previous study has found that the risk of an animal being removed as a reactor was greater at an inconclusive reactor retest (91.9 reactors per 1000 animals tested) compared to the annual test (1.5 animals per 1000 animals tested). The cause of the higher reactor rate at an inconclusive reactor retest could be either due to the animals being truly infected or to a propensity for the tester to be “risk-averse” towards these animals. The objective of this study is to follow inconclusive reactor animals over their lifetime. Three specific time periods will be examined:

- the time from the inconclusive diagnosis to the inconclusive retest;
- the results of the inconclusive retest; and
- the time from retest until slaughter or the latest SICTT result.

In particular, we will examine whether inconclusives are more likely to be slaughtered prior to the inconclusive reactor retest, have lesions following a positive inconclusive reactor retest, be sold to another herd or slaughtered in the immediate period following a negative inconclusive reactor retest, fail the SICTT at some point in the future and/or have detectable *Mycobacterium bovis* infection at slaughter.

The risk of a positive test for bovine tuberculosis in cattle purchased from herds with and without a recent history of bovine tuberculosis in Ireland

Wolfe, D.M.¹, Berke, O.¹, More, S.J.², Kelton, D.F.¹, White, P.W.^{2, 3}, O’Keeffe, J.J.^{2, 3}, Martin, S.W.¹

¹ Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada, ² UCD CVERA, ³ Department of Agriculture, Fisheries and Food

Preventive Veterinary Medicine 92, 99-105 (2009)

A retrospective cohort study was conducted to assess if cattle sold from Irish dairy herds within 7 months of herd de-restriction (clearance to trade) from a bovine-tuberculosis (BTB) episode had an excess risk of testing positive for BTB during the following 2 years, and to determine other risk factors associated with this outcome. If possible, a predictive metric for herds at high risk of selling future BTB-positive cattle would be generated. The unexposed cohort included all cattle sold within 7 months of the annual herd test in a random sample of dairy herds that did not test positive for BTB in 2003. The exposed cohort consisted of all cattle sold within 7 months of the date of de-restriction in all dairy herds that cleared a BTB episode in 2003. Only cattle sold from herds that were initially found to test positive for BTB using the single intradermal comparative tuberculin test (SICTT)—and not due to discovery of a BTB-positive animal at slaughter—were included as exposed cattle. To aid in the development of a predictive metric, the exposed cohort was subcategorized based on the number of reactors to the SICTT in the herd of origin during the BTB episode immediately prior to sale. The final exposure categories of 0 (unexposed), 1–7, and ≥ 8 total reactors were considered the unexposed, mildly exposed, and severely exposed cohorts, respectively. A multivariable logistic regression model was fit to the final BTB status of the animal using a generalized estimating equation method (GEE), assuming an exchangeable correlation structure of animals within herds, and using robust standard errors. Exposure level and the other available herd- and animal-level information were modeled. After controlling for other risk factors including the size of the herd of origin and the sex and age of the animal, the three-level exposure variable significantly improved the model (based on a change in Quasi-Akaike Information Criteria of 2.2) and demonstrated a trend of increasing risk of a future positive BTB test with increasing exposure category. The severely exposed cohort of animals had significantly higher risk of a future positive BTB test than the unexposed cohort (OR = 1.78, $p = 0.030$).

This article was published in Preventive Veterinary Medicine, 92, Wolfe, D.M., Berke, O., More, S.J., Kelton, D.F., White, P.W., O’Keeffe, J.J., Martin, S.W., The risk of a positive test for bovine tuberculosis in cattle purchased from herds with and without a recent history of bovine tuberculosis in Ireland, 99-105, Copyright Elsevier B.V. 2009.



Predictive models

From explanation to prediction: a model for recurrent bovine tuberculosis in Irish cattle herds

Wolfe, D.M.¹, Berke, O.¹, Kelton, D.F.¹, White, P.W.^{2, 3}, More, S.J.³, O'Keeffe, J.J.^{2, 3}, Martin, S.W.¹

¹ Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada,

² Department of Agriculture, Fisheries and Food, ³ UCD CVERA

Preventive Veterinary Medicine, in press

There is a good understanding of factors associated with bovine tuberculosis (BTB) risk in Irish herds. As yet, however, this knowledge has not been incorporated into predictive models with the potential for improved, risk-based surveillance. The goal of the study was to enhance the national herd scoring system for BTB risk, thus leading to improved identification of cattle herds at high risk of recurrent BTB episodes. A retrospective cohort study was conducted to develop a statistical model predictive of recurrent bovine tuberculosis episodes in cattle herds in the Republic of Ireland. Herd-level disease history data for the previous 12 years, the previous 3 years, the previous episode, and the current-episode were used in survival analyses to determine the aspects of disease history that were predictive of a recurrent breakdown within 3 years of a cleared BTB episode. Relative to herds with 0–1 standard reactors in the current BTB episode, hazard ratios increased to 1.3 and 1.6 for herds with 2–5 and >5 standard reactors, respectively. Compared to herds with <30 animals, hazard ratios increased from 1.8 to 2.5 and then to 3.1 for herds with 30–79, 80–173, and >174 animals respectively. Relative to herds with <4 herd-level tests in the previous 3 years, herds with 4–5 and >5 tests had 1.1 and 1.4 times greater hazard of a BTB breakdown. Herds that did not have a BTB episode in the 5 years prior to their 2001 episode were 0.8 times less likely to breakdown in the next three years than herds that did. Herds breaking down in the spring or summer were 0.8 times less likely to suffer a recurrent breakdown than herds breaking down in autumn or winter (this was likely due to seasonality in testing regimes). The presence of a confirmed BTB lesion was not predictive of increased risk of recurrent BTB. Despite the availability of detailed disease history, the predictive ability of the model was poor. One explanation for this was that herds suffering a recurrence of BTB on their first test after clearing a BTB episode were different from herds that broke down later in the period at risk. Future research might need to include additional variables to identify which subsets of herd BTB episodes, if any, have identifiable features that are predictive of recurrent breakdowns.

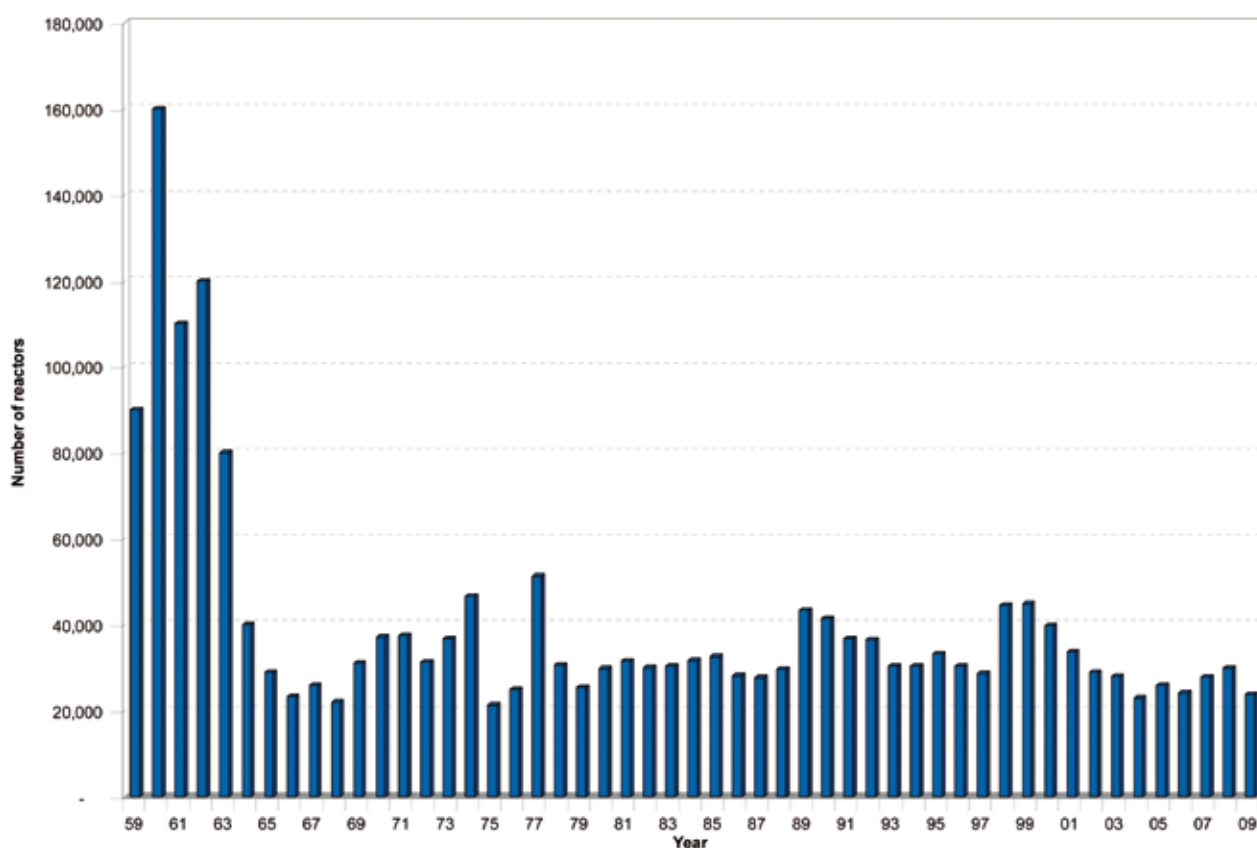
This article will be published in Preventive Veterinary Medicine, Wolfe, D.M., Berke, O., Kelton, D.F., White, P.W., More, S.J., O'Keeffe, J.J., Martin, S.W., From explanation to prediction: a model for recurrent bovine tuberculosis in Irish cattle herds, Copyright Elsevier B.V. 2010.

A case control study of temporal and spatial risk factors associated with bovine tuberculosis breakdown herds in Irish cattle herds in 2006

White, P.^{1, 2}, Frankena, K.³, O’Keeffe, J.^{1, 2}, More, S.J.¹, de Jong, M.³

¹ Department of Agriculture, Fisheries and Food, ² UCD CVERA, ³ Wageningen Institute of Animal Sciences, Wageningen University, The Netherlands

Although the temporal-spatial distribution (clustering) of BTB breakdown herds are well recognised in Ireland, and also the chronic nature of BTB infection within individual herds, little work has been published to date on the persistence of BTB infection within neighbourhoods. Using a logistic regression model, this paper aims to quantify the effect of the BTB infection status in neighbouring herds on the risk of new BTB outbreaks, while controlling for potential confounding factors. Based on 2006 test data, herds experiencing a breakdown with at minimum 1 standard reactor are classed as case herds, while herds that remained unrestricted throughout 2006 are classed as control herds. Neighbouring herds within 1 km of each study herd, will be zoned according to Euclidean distance to the nearest related fragment (within 25 m, 26-150 m, 151-500 m, and 501-1,000 m of a study herd) based on land parcel claims for 2005. BTB herd history of neighbouring herds within each zone will be assessed over the period 1989-2005 as a potential risk factor for a herd breakdown in 2006. Other herd factors will be controlled for within the model, including own herd BTB history over the period 1989-2005, purchasing history, herd size, and enterprise type. The predictive value of various competing models will be compared, and using a final model and an assessment made of the contribution of neighbourhood factors to future BTB risk.



The number of TB reactors detected in Ireland each year between 1959 and 2009.

Supporting studies

Genetics, milk production

Evidence of genetic resistance of cattle to infection with *Mycobacterium bovis*

Brotherstone, S.¹, White, I.M.S.¹, Coffey, M.², Downs, S.H.³, Mitchell, A.P.³, Clifton-Hadley, R.S.³, More, S.J.⁴, Good, M.⁵, Woolliams, J.A.⁶

¹ School of Biological Sciences, University of Edinburgh, Scotland, ² Scottish Agricultural College, Midlothian, Scotland, ³ Veterinary Laboratories Agency, Weybridge, Surrey, United Kingdom, ⁴ UCD CVERA, ⁵ Department of Agriculture, Fisheries and Food, ⁶ The Roslin Institute, Midlothian, Scotland

Journal of Dairy Science, in press

Anecdotal evidence points to genetic variation in resistance of cattle to infection with *Mycobacterium bovis*, the causative agent of bovine tuberculosis (BTB), and published experimental evidence in deer and cattle suggests significant genetic variation in resistance and reactivity to diagnostic tests. However, such genetic variation has not been properly quantified in the United Kingdom dairy cattle population and it is possible that it exists and may be a factor influencing the occurrence of BTB. Using models based on the outcome of the process of diagnosis (ultimate fate models), and on the outcome of a single stage of diagnosis (continuation ratio models, herd test-date models), this study shows that there is heritable variation in individual cow susceptibility to BTB, and that selection for milk yield is unlikely to have contributed to the current epidemic. Results demonstrate that genetics could play an important role in controlling BTB by reducing both the incidence and the severity of herd breakdowns.

This article will be published in the Journal of Dairy Science, 93, Brotherstone, S., White, I.M.S., Coffey, M., Downs, S.H., Mitchell, A.P., Clifton-Hadley, R.S., More, S.J., Good, M., Woolliams, J.A., Evidence of genetic resistance of cattle to infection with Mycobacterium bovis, 1234-1242, Copyright American Dairy Science Association, 2010.

Genetics of tuberculosis in Irish Holstein-Friesian dairy herds

Bermingham, M.L.¹, More, S.J.², Good, M.³, Cromie, A.R.⁴, Higgins, I.², Brotherstone, S.⁵, Berry, D.P.¹

¹ Teagasc Moorepark Production Research Centre, ² UCD CVERA, ³ Department of Agriculture, Fisheries and Food,

⁴ Irish Cattle Breeding Federation Society Ltd., ⁵ Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, Scotland

Journal of Dairy Science 92, 3447-3456 (2009)

Information is lacking on genetic parameters for tuberculosis (TB) susceptibility in dairy cattle. *Mycobacterium bovis* is the principal agent of tuberculosis in cattle. The objective of this study was to quantify the genetic variation present among Irish Holstein-Friesian dairy herds in their susceptibility to *M. bovis* infection. A total of 15,182 cow and 8,104 heifer single intradermal comparative tuberculin test (SICTT, a test for *M. bovis* exposure and presumed infection) records from November 1, 2002, to October 31, 2005, were available for inclusion in the analysis. Data on observed carcass TB lesions from abattoirs were also available for inclusion in the analysis. The only animals retained were those present in a herd during episodes in which at least 2 animals showed evidence of infection; this ensured a high likelihood of exposure to *M. bovis*. Linear animal models, and sire and animal threshold models were used to estimate the variance components for susceptibility to *M. bovis*-purified protein derivative (PPD) responsiveness and confirmed *M. bovis* infection. The heritability estimates from the threshold sire models were biased upward because the relatedness between dam-daughter pairs was ignored. The threshold animal model produced heritability estimates of 0.14 in cows and 0.12 in heifers for susceptibility to *M. bovis*-PPD responsiveness, and 0.18 in cows for confirmed *M. bovis* infection susceptibility. Therefore, exploitable genetic variation exists among Irish dairy cows for susceptibility to *M. bovis* infection. Sire

rankings from the linear and threshold animal models were similar, indicating that either model could be used for the analysis of susceptibility to *M. bovis*-PPD responsiveness. A favorable genetic correlation close to unity was observed between susceptibility to confirmed *M. bovis* infection and *M. bovis*-PPD responsiveness, indicating that direct selection for resistance to *M. bovis*-PPD responsiveness will indirectly reduce susceptibility to confirmed *M. bovis* infection. Data from the national TB eradication program could be used routinely to estimate breeding values for susceptibility to *M. bovis* infection.

This article was published in Journal of Dairy Science, 92, Bermingham, M.L., More, S.J., Good, M., Cromie, A.R., Higgins, I., Brotherstone, S., Berry, D.P., Genetics of tuberculosis in Irish Holstein-Friesian dairy herds, 3447-3456, Copyright American Dairy Science Association, 2009.

Genetic correlations between measures of *Mycobacterium bovis* infection and economically important traits in Irish Holstein-Friesian dairy cows

Bermingham, M.L.¹, More, S.J.², Good, M.³, Cromie, A.R.⁴, Higgins, I.M.², Berry, D.P.¹

¹ Teagasc Moorepark Production Research Centre, ² UCD CVERA, ³ Department of Agriculture, Fisheries and Food,

⁴ Irish Cattle Breeding Federation Society Ltd.

Previous research has shown that there is considerable genetic variation for susceptibility to the measures of *Mycobacterium bovis* infection, confirmed *M. bovis* infection and *M. bovis*-purified protein derivative (PPD) responsiveness in Irish Holstein-Friesian dairy cattle. The objective of this study was to estimate the genetic and phenotypic correlations between economically important traits and these measures of *M. bovis* infection. A total of 20,148 and 17,178 cows with confirmed *M. bovis* infection and *M. bovis*-PPD responsiveness records respectively were available for inclusion in the analysis. First to third parity milk, fat, and protein yield, somatic cell count, calving interval and survival, as well as first parity body condition score records were available on cows calving between 1985 and 2007. Bivariate linear sire mixed models were used to estimate (co)variance components. The genetic correlations between susceptibility to confirmed *M. bovis* infection and economically important traits investigated in this study were all close to zero. Susceptibility to *M. bovis*-PPD responsiveness was positively genetically correlated with fat production (0.39) and body condition score (0.36), and negatively correlated with somatic cell score (-0.34) and survival (-0.62). Hence, selection for increased survival may indirectly reduce susceptibility to *M. bovis* infection, while selection for reduced somatic cell count and increased fat production and body condition score may increase susceptibility to *M. bovis* infection.

Bovine tuberculosis and milk production in infected dairy herds in Ireland

Boland, F.¹, Kelly, G.E.¹, Good, M.², More, S.J.³

¹ UCD School of Mathematical Sciences, ² Department of Agriculture, Fisheries and Food, ³ UCD CVERA

Preventive Veterinary Medicine, in press

This study describes the relationship between bovine tuberculosis (TB) and milk yield in TB-infected dairy herds in Ireland. The study had two objectives: to determine whether cows detected as TB reactors (and thus subject to immediate slaughter) were likely to be the higher milk-producing cows, and to determine whether subclinical TB infection was associated with reduced milk production at or around the time of disclosure (detection). All Irish dairy herds restricted from trading between the 1st June 2004 and the 31st May 2005 as a result of two or more TB reactors by the Single Intradermal Comparative Tuberculin Test (SICTT) were considered for study. The data consisted of 419 herds. Data were collected on all TB reactors and a random sample of 5 non-reactor cows in these herds: a data set of 4340 cows (2342 TB reactors and 1998 non-reactors). Previous milk data for the cows were taken into consideration and thus all lactations on a cow were analysed together with the years of lactations. There was an inherent hierarchical structure in the data, with lactations nested within cows and cows within herds and thus a linear mixed model with two random effects was used to describe the data. The results of this study showed that for all lactations and years under investigation, milk yield was significantly lower for TB reactor cows, with differences ranging from 120 kg (2003, lactation 3) to 573 kg (2001, lactation 1), when compared to the non-reactor cows.

This article will be published in Preventive Veterinary Medicine, 93, Boland, F., Kelly, G.E., Good, M., More, S.J., Bovine tuberculosis and milk production in infected dairy herds in Ireland, 153-161, Copyright Elsevier B.V. 2010.

Tuberculosis in other farmed livestock species

Control of *Mycobacterium bovis* infection in two sika deer herds in Ireland

Partridge, T.¹, Toolan, D.², Egan, J.¹, More, S.J.³

¹ Department of Agriculture, Fisheries and Food, ² Kilkenny Regional Veterinary Laboratory, Department of Agriculture, Fisheries and Food, ³ UCD CVERA

Irish Veterinary Journal 61, 27-32 (2008)

In a number of countries, tuberculosis (due to infection with *Mycobacterium bovis*) is a significant health problem of captive deer. This paper describes outbreaks of bovine tuberculosis in sika deer (*Cervus nippon*) on two farms in Ireland and the methods used to control the disease. On Farm A, infection was first detected during 1993. The infection was eradicated using a programme of test and removal, in association with segregation of young animals. A second outbreak (also due to infection with *M. bovis*, but a different RFLP profile) was detected in 2002. In the latter outbreak, infection was particularly prevalent in two groups of young deer. *M. bovis* with the same RFLP profile was also isolated in a badger found dead on the farm. Control was achieved by test and removal in association with herd management changes. In Herd B, infection was first detected in 1995, and subsequently eradicated using test and removal alone. In herd A, re-infection remains an ongoing risk. Control rather than eradication of infection may be more realistic in the short- to medium-term.

Printed with permission from the Irish Veterinary Journal.

Tuberculosis in alpaca (*Lama pacos*) on a farm in Ireland. 1. A clinical report

Ryan, E.G.¹, Dwyer, P.J.², Connolly, D.J.³, Fagan, J.², Costello, E.⁴, More, S.J.⁵

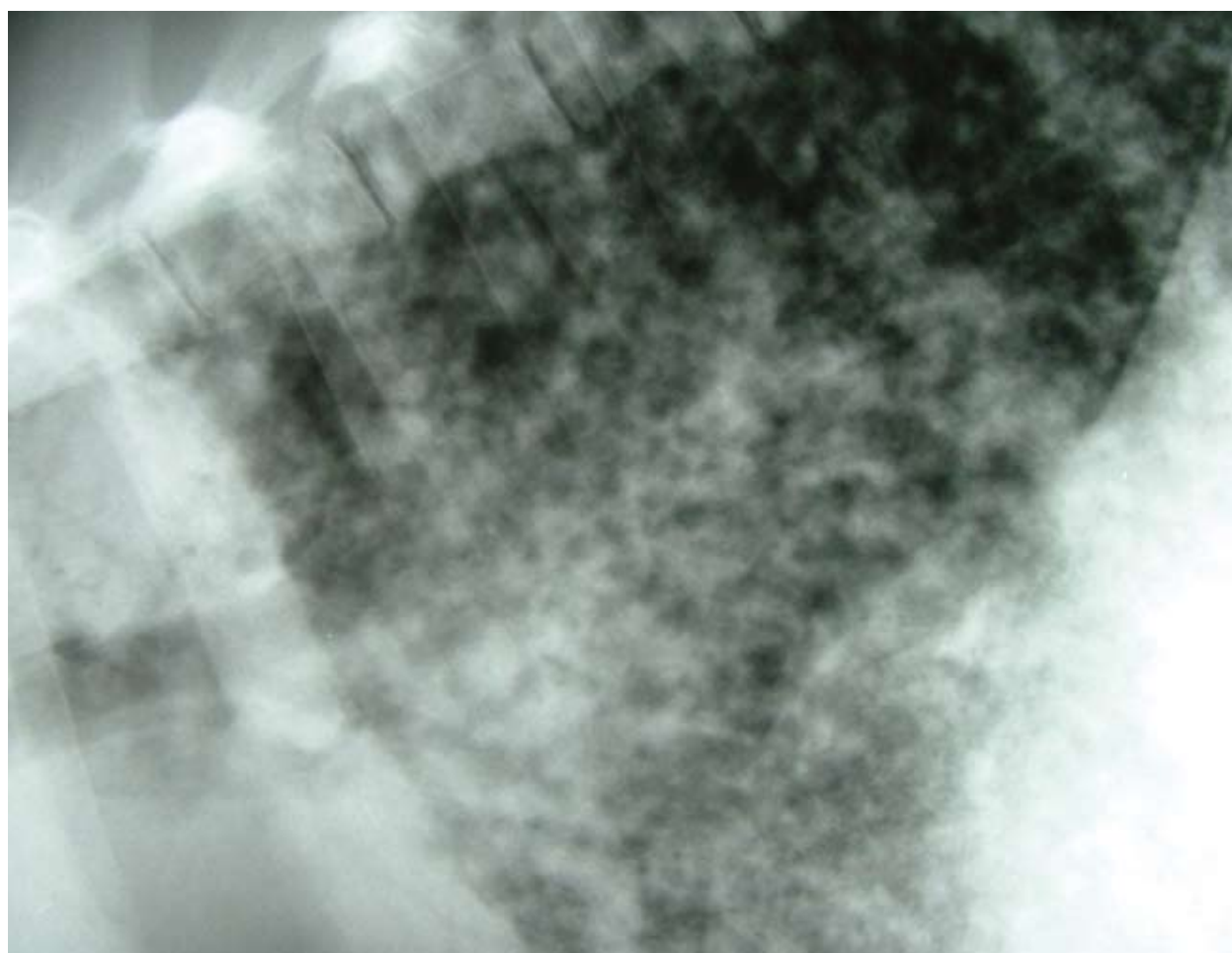
¹ Private veterinary practitioner, ² Athlone Regional Veterinary Laboratory, Department of Agriculture, Fisheries and Food,

³ Private consultant, ⁴ Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food, ⁵ UCD CVERA

Irish Veterinary Journal 61, 527-531 (2008)

This case report describes tuberculosis (TB) due to infection with *Mycobacterium bovis* in alpaca (*Lama pacos*) on a farm in Ireland. Two severely debilitated alpaca were presented to the University Veterinary Hospital, University College Dublin in November 2004. Bloods were taken, and haematology and biochemistry results were indicative of chronic infection. Radiological examination showed evidence of diffuse granulomatous pneumonia suggestive of tuberculosis. On necropsy there were granulomatous lesions present throughout many body organs including lung, liver, kidney, intestine as well on peritoneum and mesentery. Culture of acid-fast bacilli from lesions led to a diagnosis of tuberculosis due to *M. bovis*. The use of intradermal skin testing proved inefficient and unreliable for ante mortem diagnosis of tuberculosis in alpaca. Infection due to *M. bovis* should be considered among the differential diagnoses of debilitating diseases in alpaca, particularly those farmed in areas known to be traditional black spots for tuberculosis in cattle.

Printed with permission from the Irish Veterinary Journal.



Lateral radiograph (x-ray) from an alpaca with pulmonary tuberculosis. Radiograph taken at the UCD Veterinary Hospital.

Tuberculosis in alpaca (*Lama pacos*) on a farm in Ireland. 2. Results of an epidemiological investigation

Connolly, D.J.¹, Dwyer, P.J.², Fagan, J.², Hayes, M.³, Ryan, E.G.⁴, Costello, E.⁵, Kilroy, A.³, More, S.J.⁶

¹ Private consultant, ² Athlone Regional Veterinary Laboratory, Department of Agriculture, Fisheries and Food, ³ Department of Agriculture, Fisheries and Food, ⁴ UCD School of Agriculture, Food Science and Veterinary Medicine, ⁵ Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food, ⁶ UCD CVERA

Irish Veterinary Journal 61, 533-537 (2008)

Tuberculosis (TB), due to infection with *Mycobacterium bovis* was diagnosed in a flock of alpaca in Ireland in 2004. An epidemiological investigation was conducted to identify the risk of TB for farmed alpaca where TB is endemic, the origin of the infection, the potential for alpaca-to-alpaca transmission and appropriate control measures. The investigation focused on the alpaca flock (including the farm, animal movements and breeding, feeding and flock health practice), the disease episode (including animal disease events and subsequent control measures) and TB infection risk in the locality. The TB risk to alpaca is high in areas where infection is endemic in cattle and badgers and where biosecurity is inadequate. It is most likely that the source of infection for the alpaca was a local strain of *M. bovis*, present in cattle in this area since at least 2001. Genotyping of isolates identified a single variable number tandem repeat (VNTR) profile in both cattle and alpaca in this region. Although a tuberculous badger was also removed from the vicinity, bacterial isolation was not attempted. On this farm, infection in alpaca was probably derived from a common source. Alpaca-to-alpaca transmission seems unlikely. Two broad control strategies were implemented, aimed at the rapid removal of infected (and potentially infectious) animals and the implementation of measures to limit transmission. Tests that proved useful in detecting potentially-infected animals included measurement of the albumin-to-globulin ratio and regular body condition scoring. Skin testing was time consuming and unproductive, and early detection of infected animals remains a challenge. The flock was managed as a series of separate groupings, based on perceived infection risk. No further TB cases have been detected.

Printed with permission from the Irish Veterinary Journal.

Tuberculosis in goats on a farm in Ireland: epidemiological investigation and control

Shanahan, A.¹, Good, M.¹, Duignan, A.^{1, 2}, Curtin, T.¹, More, S.J.²

¹ Department of Agriculture, Fisheries and Food, ² UCD CVERA

In a number of countries, bovine tuberculosis (BTB; due to infection with *Mycobacterium bovis*) is a significant health problem of farmed goats. However, few case reports are available. In Ireland, BTB is a problem in the national cattle herd, and is endemic in badgers (*Meles meles*). Infection has also been periodically confirmed in goats. Under EU Regulation 853/2004/EC, Ireland requires food business operators collecting or producing raw milk from species susceptible to BTB, which includes goats, to have a control plan for tuberculosis in place and approved by the competent Authority. This paper describes an outbreak of bovine tuberculosis in a dairy goat herd on a farm in Ireland, where 66.3% of the herd was SICTT-positive at initial detection. An epidemiological investigation was conducted to determine the origin of the outbreak. The investigation considered a range of issues including animal movements and herd management practices. Infection was introduced to the holding with a consignment of goats as determined by the VNTR (variable number tandem repeat) profile. The infection was eradicated using a programme of test and removal involving the SICTT (single intradermal comparative tuberculin test), the γ -interferon assay and a multiplex immunoassay (Enferplex TB). These tests are still at development stage for use in goats in Ireland. There was good correlation between tests, and infection was rapidly cleared from this heavily infected herd.



Alpaca, the west of Ireland. Photograph by S.J. More.

Improved understanding of ecology and TB epidemiology

Badger ecology

How many Eurasian badgers *Meles meles* L. are there in the Republic of Ireland?

Sleeman, D.P.¹, Davenport, J.¹, More, S.J.², Clegg, T.A.², Collins, J.D.², Martin, S.W.³, Williams, D.H.⁴, Griffin, J.M.⁵, O'Boyle, I.⁵

¹ Department of Zoology, Ecology and Plant Science, University College Cork, ² UCD CVERA, ³ Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada, ⁴ UCD School of Mathematical Sciences, ⁵ Department of Agriculture, Fisheries and Food

European Journal of Wildlife Research 55, 333-344 (2009)

In Ireland, the badger *Meles meles* L. is a reservoir species for *Mycobacterium bovis* and, as such, contributes to the maintenance of bovine tuberculosis in cattle. A previous estimate of the badger population in the Republic was 200,000 badgers. In the current study, we obtained data on badger numbers from a large-scale badger removal project (the Four-Area project). The removal areas of the Four-Area Project were surrounded by barriers (either water or buffer areas where removals were also conducted) to prevent badger immigration. Within these areas, a grid of 0.25 km² was created within which we knew the badger numbers and habitat types (based on Corine data). Associations between badger numbers and habitat type were investigated using negative binomial modeling. Extrapolations from the model yielded an estimated badger population in the Republic of approximately 84,000 badgers. The implications of these findings are discussed.

With kind permission of Springer Science+Business Media.

The full paper can be found at: <http://dx.doi.org/10.1007/s10344-008-0244-1>



The effectiveness of barriers to badger *Meles meles* immigration in the Irish four area project

Sleeman, D.P.¹, Davenport, J.¹, More, S.J.², Clegg, T.A.², Griffin, J.M.³, O'Boyle, I.³

¹ Department of Zoology, Ecology and Plant Science, University College Cork, ² UCD CVERA, ³ Department of Agriculture, Fisheries and Food

European Journal of Wildlife Research 55, 267-278 (2009)

This study's objective was to estimate the permeability of barriers to badger immigration during the Irish Four Area project. These barriers were at the boundaries of removal areas, where there was proactive culling of badgers. Data from the last 3 years of the study were used. Each length of barrier was allocated a space within the removal area. These were further sub-divided into spaces of 0–2, 2–5 km and sometimes of more than 5 km from the edge of the removal area. It is assumed that all, or some, of the badgers caught within these spaces came across the barriers. The barriers were one of the following: external buffers, sea, rivers and political boundaries. The total lengths of the barriers in all areas were: external buffer 128.5 km; sea 70.9 km; river 78.6 km; political 32.2 km. We assume three scenarios: (1) all badgers caught in the final 3 years were immigrants, (2) 75% were immigrants or (3) 50% were immigrants. We test these scenarios using chi-square tests, applying internal buffers of 1 km to counter movements of badgers across zones. Using this approach and multivariate analysis, we found that the permeability of barrier types varied, with sea and external buffers being the most effective barriers. The combined capture data are further examined by the sex ratio in each range, and then the sex ratio in total. Equal numbers of males and females were found, but the source populations were probably predominantly female. If badger management options are to achieve maximum benefits, then the field effectiveness of such barriers needs to be understood.

With kind permission of Springer Science+Business Media.

The full paper can be found at: <http://dx.doi.org/10.1007/s10344-008-0241-4>

The small-bodied badgers (*Meles meles* L.) of Rutland Island, Co. Donegal

Sleeman, D.P.¹, Davenport, J.¹, Cussen, R.E.¹, Hammond, R.F.²

¹ Department of Zoology, Ecology and Plant Science, University College Cork, ² UCD CVERA

Irish Naturalists' Journal 30, 1-6 (2009)

Badgers found on an island, Rutland Island, Co. Donegal were investigated. When compared with badgers from the Donegal mainland they are significantly smaller in both length and weight. Twenty-eight setts were found in a survey in 1998 and fifteen Badgers were trapped in 1999. GPS was used to map and re-locate setts and latrines. Bait-marking in 2003 showed that there were two social groups on the island at that time. The results are discussed with reference to the badgers' adaptation to the island, their population structure, use of setts and the research opportunities, for both badger management and conservation, on the island.

Printed with permission from The Irish Naturalists' Journal Ltd.

The diet of the badger *Meles meles* in the Republic of Ireland

Cleary, G.P.¹, Corner, L.A.L.², O'Keeffe, J.^{3, 4}, Marples, N.M.¹

¹ School of Natural Sciences, Trinity College Dublin, ² UCD TB Diagnostics and Immunology Research Centre,

³ Department of Agriculture, Fisheries and Food, ⁴ UCD CVERA

Mammalian Biology 74, 438-447 (2009)

The diet of the Eurasian badger (*Meles meles*) in the Republic of Ireland was studied by examination of the stomach of the contents of 686 badgers, collected between March 2005 and September 2006. It was found that the relative importance of different food types, as indicated by their frequency of occurrence and ingested bulk in the diet, fluctuated seasonally. Tipulid larvae (Cl. Insecta, Ord. Tipulidae) dominated the diet in spring; Anura (Cl. Amphibia) and Aculeata (Ord. Hymenoptera) during the summer; and Noctuid larvae (Cl. Insecta, Ord. Noctuidae) in autumn and winter. Thus this type of foraging behaviour supports the contention that badgers are generalist foragers with seasonal food preferences. This feeding behaviour is more similar to that of badgers in Italy and Spain than to badgers in England.

This article was published in Mammalian Biology, 74, Cleary, G.P., Corner, L.A.L., O'Keeffe, J., Marples, N.M., The diet of the badger Meles meles in the Republic of Ireland, 438-447, Deutsche Gesellschaft für Säugetierkunde. Published by Elsevier GmbH. 2009.

Diet of the badger (*Meles meles*) in the Republic of Ireland: a comparison of results from an analysis of stomach contents and rectal faeces

Cleary, G.P.¹, Corner, L.A.L.², O'Keeffe, J.^{3, 4}, Marples, N.M.¹

¹ School of Natural Sciences, Trinity College Dublin, ² UCD TB Diagnostics and Immunology Research Centre,

³ Department of Agriculture, Fisheries and Food, ⁴ UCD CVERA

The diets of mammals have been investigated primarily through the analysis of faecal samples. In our study we analysed both stomach contents, and rectal faeces from European badgers. This approach enabled a direct comparison of the information derived from these two sources. The dietary components found from each source were the same. However, it was found that, compared to stomach contents, the contribution to the diet, by volume, of plant litter, earthworms, Tipulid larvae and adult Carabid beetles were significantly overestimated by faecal analysis, while those of Noctuid larvae and Carabid beetle larvae were significantly underestimated. The analysis of stomach contents showed clear evidence of seasonality in the consumption of earthworms, Carabid beetle larvae, Tipulid larvae and Noctuid larvae. This seasonality was not as evident when the diet was inferred by the analysis of faeces. We propose that an analysis of stomach contents rather than of faeces, more accurately reflects the relative proportions of ingested food types, and the seasonality of the diet.

The reproductive cycle of the male and female Eurasian badger

Stuart, L.¹, Corner, L.A.L.², O’Keeffe, J.^{3, 4}, Marples, N.M.¹

¹ School of Natural Sciences, Trinity College Dublin, ² UCD TB Diagnostics and Immunology Research Centre,

³ Department of Agriculture, Fisheries and Food, ⁴ UCD CVERA

The nationwide control programme of reactive badger removal provided an excellent opportunity to detail the reproductive cycle of the male and female badger in Ireland, as a comparison to previous studies from England and parts of mainland Europe. Badgers were obtained from a geographically wide range of sites in Ireland during all months of the year, making it possible to describe the annual reproductive cycle of the male and female badger in full, utilising a large sample size. The reproductive tracts were obtained from post mortem examinations of the badger. Both gross examination and in-depth histological examinations were carried out on all tissues; assays were conducted to determine individual levels of reproductive hormones. A number of demographic measurements were also taken to help with identification of trends or varying population dynamics within the study population. The badger population in Ireland is of low-medium density consisting of small social groups. The lack of aggression observed suggests that there may be high levels of tolerance both within and between social groups; possibly owing to small group sizes, high levels of philopatry, inter-group relatedness, and relatively low levels of testosterone. Mate guarding behaviour may be abandoned due to high levels of philopatry or an inability to monopolise paternity. A comparison of male and female badgers suggests that each has adopted a very different breeding strategy. The majority of males show a breeding pattern that is reminiscent of a seasonal breeder, with high fertility associated with the early oestrous cycles of the female in Feb-April and declining fertility for the remainder of the year. By contrast the female has adopted a strategy whereby they are lactating during a period of nutritional abundance in Feb-March, and cubs are also weaned during favourable conditions. This is achievable due to the long period of delayed implantation employed by this species, such that implantation takes place in Dec-Jan. Badgers have continued oestrous cycles throughout the breeding year, providing replacement or additional blastocysts, which increases the probability of successful conception at the end of the period of delay. Furthermore, continued oestrous cycles may provide additional corpora lutea as a source of progesterone, needed to sustain the diapausing blastocysts. This female strategy also increases the probability of superfetation and polyandry which leads to an increase in female fitness and increased cub survival. Approximately 40% of mature females bred successfully and as the number of blastocysts per female was similar to the number of foetuses, the potential was maintained through to parturition. Failure at the fertilisation stage of the reproductive cycle was responsible for the greatest losses to reproductive potential.



The effects of culling on the badger (*Meles meles*) population in Ireland

Carroll, R.¹, Corner, L.A.L.², Marples, N.M.¹

¹ School of Natural Sciences, Trinity College Dublin, ² UCD TB Diagnostics and Immunology Research Centre

Since the interim ban on culling implemented by the Department of Agriculture, Food and Fisheries (DAFF) is set to continue, it is essential that it takes place at the most appropriate time of year. If not then there is a danger of suckling females being captured when they still have dependent cubs, thus leaving the cubs to starve. When determining the most opportune time for this closed season to take place it is important to establish whether cubbing occurs at the same time each year and if not, then what factors affect the birthing dates.

The information gathered from the postmortems carried out on these badgers enabled us to describe the cubbing season of badgers in Ireland, and so prevent the culling of suckling sows. Therefore the first aim of this project was to predict the annual cubbing trends in Ireland and investigate the differences in social group size and reproductive success found between areas of previously undisturbed groups and newly established groups. The second aim was to investigate whether the bovine TB (bTB) eradication scheme has changed the badger population in Ireland with regard to social group organisation and reproductive potential. We further aimed to investigate social group dynamics, focusing mainly on sett densities and usage, and recovery times after culling. Finally we hope to develop a methodology which will successfully age a subsection of the Irish badger population, using teeth sectioning and x-ray imaging techniques, using both known and unknown age jaws.

Incidence of visits by badgers to farmyards in Ireland in winter

Sleeman, D.P.¹, Davenport, J.¹, Fitzgerald, A.²

¹ Department of Zoology, Ecology and Plant Science, University College Cork, ² Department of Epidemiology and Public Health, University College Cork

Veterinary Record 163, 734 (2008)

Visits by infected badgers to yards where cattle are housed in winter may provide opportunities for transmission of tuberculosis to cattle. Using winter survey periods in 2005/06 and 2006/07, this study quantified the badger activity in cattle yards in Co. Cork. It was found to be very rare.

Printed with permission from BVA Publications.

Mammalian road casualties in the Cork four area project

O'Shea, F.¹, Sleeman, D.P.², Davenport, J.²

¹ Department of Agriculture, Fisheries and Food, ² Department of Zoology, Ecology and Plant Science, University College Cork

Records of a five year survey of mammalian road casualties in two different parts (removal and reference areas) of the County Cork portion of the Four Area Badger Project are reported. There were more hedgehog, cat and fox casualties in the removal area, but only the first two species differed to a statistically significant extent from the reference area. How such studies might be improved and the possible implications for epidemiology and biodiversity are discussed.

TB epidemiology

Estimating the extent of spatial association of *Mycobacterium bovis* infection in badgers in Ireland

Kelly, G.E.¹, McGrath, G.E.², More, S.J.²

¹ School of Mathematical Sciences, University College Dublin, ² UCD CVERA

Epidemiology and Infection, in press

Mycobacterium bovis infects the wildlife species badgers *Meles meles* who are linked with the spread of the associated disease tuberculosis (TB) in cattle. Control of livestock infections depends in part on the spatial and social structure of the wildlife host. Here we describe spatial association of *M. bovis* infection in a badger population using data from the first year of the Four Area Project in Ireland. Using second-order intensity functions, we show there is strong evidence of clustering of TB cases in each the four areas, i.e. a global tendency for infected cases to occur near other infected cases. Using estimated intensity functions, we identify locations where particular strains of TB cluster. Generalized linear geo-statistical models are used to assess the practical range at which spatial correlation occurs and is found to exceed 6 in all areas. The study is of relevance concerning the scale of localized badger culling in the control of the disease in cattle.

Printed with permission from Cambridge University Press. *Epidemiology and Infection* 138, 270-279 (2010).

The prevalence and distribution of *Mycobacterium bovis* infection in European badgers (*Meles meles*) as determined by enhanced post mortem examination and bacteriological culture

Murphy, D.¹, Gormley, E.¹, Costello, E.², O'Meara, D.², Corner, L.A.L.¹

¹ UCD TB Diagnostics and Immunology Research Centre, ² Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food

Research in Veterinary Science, in press

The accurate diagnosis of *Mycobacterium bovis* infection in badgers is key to understanding the epidemiology of tuberculosis in this species and has significant implications for devising strategies to limit spread of the disease. In this study, badgers ($n = 215$) in the Republic of Ireland were examined at post mortem and tissues were collected from a range of anatomical locations and pooled into groups for bacterial culture of *M. bovis*. By assessing confirmed gross visible lesions (VL) alone, infection was detected in 12.1% of badgers. However, by including the results of all culture positive pooled samples, the overall infection prevalence increased significantly to 36.3%. Two-thirds (66.7%) of infected animals had no visible lesions (NVL). While the thoracic cavity (lungs and pulmonary lymph nodes) was found to be the most common site of infection, in a proportion of animals infection was absent from the lungs and draining lymph nodes and was confined to the lymph nodes of the carcass or the head. This may indicate an early extra-pulmonary dissemination of infection or alternatively, in the case of the head lymph nodes, a secondary pathogenic pathway involving the lymphoid tissues of the upper respiratory tract (URT).

This article will be published in *Research in Veterinary Science*, 88, Murphy, D., Gormley, E., Costello, E., O'Meara, D., Corner, L.A.L., *The prevalence and distribution of Mycobacterium bovis infection in European badgers (Meles meles) as determined by enhanced post mortem examination and bacteriological culture*, 1-5, Copyright Elsevier Ltd. 2010.

Tuberculosis in cattle herds are sentinels for *Mycobacterium bovis* infection in European badgers (*Meles meles*): the Irish greenfield study

Murphy, D.¹, Gormley, E.¹, Collins, D.M.², McGrath, G.², Sorvic, E.³, Costello, E.³, Corner, L.A.L.³

¹ UCD TB Diagnostics and Immunology Research Centre, ² UCD CVERA,

³ Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food

In Ireland badgers are removed in response to tuberculosis breakdowns in cattle herds (focal culling). Prevalence studies, conducted using a detailed post mortem and bacteriological examination, showed that 36-50% of badgers were infected with *Mycobacterium bovis*. Focal culling forms part of the medium term national strategy for the control of bovine TB in cattle and is based on the premise that badgers in areas with herd breakdowns have a higher prevalence of infection than the badger population at large. However, the hypothesis that cattle can be used as sentinels for infection in the badger population has never been formally tested. In this study we tested the hypothesis by determining the infection prevalence in badgers in areas where there had been historically, a consistently low prevalence of infection in cattle. Low cattle TB prevalence areas were defined as those herds with ≤ 2 standard reactors in the annual round of skin testing over the preceding 5 years (Greenfield sites). Using GIS, and adjusting for variation in land use, previous culling and cattle density, 198 Greenfield sites were identified and surveyed, and 138 areas with badger setts or signs of badger activity were identified. A single badger was removed from 87 sites and all were examined using detailed post mortem and bacteriological procedures. A prevalence of *M. bovis* infection of 14.9% was found in the Greenfield site badgers. This prevalence was significantly lower ($P < 0.001$) than in badgers removed during focal culling (36.6%). The results validate the use of cattle as sentinels for TB in badgers and support the medium term national strategy for the control of bovine TB. The geographic variation in prevalence in the Irish badger populations will be used when devising strategies for the incorporation of badger vaccination into the long term bovine TB control programme.

The Irish redfield study

Murphy, D.¹, Gormley, E.¹, Collins, D.M.², McGrath, G.², Sorvic, E.³, Costello, E.³, Corner, L.A.L.³

¹ UCD TB Diagnostics and Immunology Research Centre, ² UCD CVERA,

³ Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food

In parallel to the Greenfield study, a study has been conducted of badgers removed from routine first event culls associated with herd breakdowns. The Redfield study examines the infection prevalence in badgers removed in the first trapping event in areas of high prevalence of infection in cattle. Bacterial culture results are pending. While not directly comparable due to different selection criteria, the results of the studies will identify any significant difference in the prevalence of infection in the two badger populations.

Spatial clustering of TB-infected cattle herds in Ireland prior to and following pro-active badger removal

Kelly, G.E.¹, More, S.J.²

¹ School of Mathematical Sciences, University College Dublin, ² UCD CVERA

Bovine tuberculosis (TB; caused by infection with *Mycobacterium bovis*) is primarily a disease of cattle. In both Ireland and the UK, badgers (*Meles meles*) contribute to the epidemiology of infection in cattle. Control of infection depends in part on our understanding of the spatial structure of the disease. Data from the Four Area Project, a large-scale intervention study aimed at assessing the effect of proactive badger culling on bovine TB incidence in cattle herds, are analyzed for the first time using logistic models that explicitly include spatial random effects i.e. generalized linear geostatistical models. We establish that infected herds are spatially correlated (the scale of spatial correlation is presented), but at a scale that varies with time and in different areas. Spatial correlation is shown to persist following proactive badger removal, consistent with ongoing residual herd infection. The results are useful in informing TB control policy.



Infection control strategies

Badger removal

Controlling wildlife reservoirs for bovine TB

Hewinson, G.¹, Buddle, B.², More, S.J.³, Clifton-Hadley, R.¹

¹ Veterinary Laboratories Agency, Weybridge, Surrey, England, ² AgResearch, Hopkirk Research Institute, Palmerston North, New Zealand, ³ UCD CVERA

Bovine tuberculosis (bTB) is caused by *Mycobacterium bovis* and closely related members of the *M. tuberculosis*-complex. These organisms have an extensive host range that includes bovines, other livestock including small ruminants such as goats, a wide range of wildlife and humans. Eradication programmes based on cattle test-and-slaughter policy have proved successful in some countries but have failed to eradicate disease in others due, at least in part, to the presence of reservoirs of TB in wildlife. There are three main approaches to controlling the spread of TB from wildlife to livestock: keeping livestock and wildlife apart, culling or vaccination. However, the control of tuberculosis in wildlife is a complex process and involves an understanding of the mechanisms of transmission of *M. bovis* between wildlife, and from wildlife to livestock, as well as the ecology, biology and behavior of the wildlife reservoir. Without such knowledge implementation of control strategies can lead to unexpected consequences. Approaches to the control of TB in wildlife reservoirs will be discussed in the context of the successful Australian TB eradication scheme, TB control in possums in New Zealand and the badger culling trials that have been conducted in Great Britain.

The effect of varying levels of population control on the prevalence of tuberculosis in badgers in Ireland

Corner, L.A.L.¹, Clegg, T.A.², More, S.J.², Williams, D.H.³, O'Boyle, I.⁴, Costello, E.⁵, Sleeman, D.P.⁶, Griffin, J.M.⁴

¹ UCD TB Diagnostics and Immunology Research Centre, ² UCD CVERA, ³ School of Mathematical Sciences, University College Dublin,

⁴ Department of Agriculture, Fisheries and Food, ⁵ Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food,

⁶ Department of Zoology, Ecology and Plant Science, University College Cork

Research in Veterinary Science 85, 238-249 (2008)

We examined the effect of varying levels of badger population control on the prevalence of *Mycobacterium bovis* infection in badgers in four counties of Ireland. In the 'Removal' and 'Buffer' areas, proactive culling was conducted to substantially reduce and subsequently maintain badger populations at a low level for five years. In the 'Reference' areas, localised reactive culling was conducted in association with herd breakdowns. The infection status of badgers was determined using bacteriology. A total of 2,696 badgers were recruited into the study, and 19.0% were found to be infected with *M. bovis*. The two population control strategies had differing effects on the subsequent prevalence of tuberculosis in badger populations. Proactive culling led to a long term decrease in the prevalence of tuberculosis in the re-emergent populations. Although there was an overall decline in the disease prevalence, no consistent trend in disease prevalence as a result of reactive culling was observed.

This article was published in Research in Veterinary Science, 85, Corner, L.A.L., Clegg, T.A., More, S.J., Williams, D.H., O'Boyle, I., Costello, E., Sleeman, D.P., Griffin, J.M., The effect of varying levels of population control on the prevalence of tuberculosis in badgers in Ireland, 238-249, Copyright Elsevier Ltd. 2008.

A long-term observational study of the impact of badger removal on herd restrictions due to bovine TB in the Irish midlands during 1989–2004

Kelly, G.E.¹, Condon, J.², More, S.J.³, Dolan, L.⁴, Higgins, I.³, Eves, J.⁴

¹ School of Mathematical Sciences, University College Dublin, ² School of Mathematical Sciences, Dublin Institute of Technology,

³ UCD CVERA, ⁴ Department of Agriculture, Fisheries and Food

Epidemiology and Infection 136, 1362-1373 (2008)

An observational study was carried out, using data collected from four areas in the Irish midlands, between 1989 and 2004, to critically evaluate the long-term effects of proactive badger culling and to provide insights into reactive badger culling tuberculosis (TB) prevalence in cattle. Confirmed cattle herd TB incidence is the outcome measure used throughout. Relative to reactive culling, proactive badger culling was associated with a decrease in incidence in each of the 16 years of observation, which encompassed periods of both intensive and less-intensive badger removal. By 2004, we observed a decrease of 22% [95% confidence interval (CI) 15–29, $P<0.001$] in the entire proactive and 37% (95% CI 25–47, $P<0.001$), in the inner proactive removal areas. The size of the decrease increased with time ($P=0.055$). There was a decrease (constant over time) of at least 14% (95% CI 76–97, $P=0.013$) in incidence in the inner compared to the outer control area (herds ≤ 2 km, > 2 km, from proactive removal area boundaries, respectively). Incidence in the outer proactive removal area (herds < 1.6 km from the proactive removal boundary) was similar to the inner control area ($P=0.890$). Incidence in the outer control area and total control area, compared to a neighbouring area some distance away, increased over the course of the study. Differences with the total control area were not statistically significant but the outer control area was 11% higher than the neighbouring area by 2004 (borderline significance $P=0.057$).

Printed with permission from Cambridge University Press.

Targeted badger removal and the subsequent risk of bovine tuberculosis in cattle herds in county Laois, Ireland

Olea-Popelka, F.P.^{1, 2}, Fitzgerald, P.³, White, P.^{3, 4}, McGrath, G.⁴, Collins, J.D.⁴, O’Keeffe, J.^{3, 4}, Kelton, D.F.¹, Berke, O.¹, More, S.J.⁴, Martin S.W.¹

¹ Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada, ² Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Colorado, USA, ³ Department of Agriculture, Fisheries and Food, ⁴ UCD CVERA

Preventive Veterinary Medicine 88, 178-184 (2009)

We investigated the impact of targeted removal of badgers on the subsequent bovine tuberculosis (BTB) risk in cattle herds in county Laois, Ireland. The study period was 1989–2005. For each of 122 targeted badger-removal licenses (permit to remove badgers in the proximity of cattle herds undergoing a serious BTB episode), the herd number (index herd) for which the license was given was obtained. The herds in the proximity of the index herd were identified from another database. The main “exposure” in our study was the geographical location of herds relative to the area in which targeted badger removal was conducted. We categorized herds into five different exposure groups: herds were classified as non-exposed and denoted as group 0 (reference group) if they were located 500 m or more from the edge of any parcel of land of the index herd; group 1, was the index herds, group 2 the immediate (contiguous) neighbors of the index herd, group 3 herds were not immediate neighbors but within 150 m and group 4 herds were between 150 m and 500 m distance from the edge of any parcel of land of the index herd, respectively. We conducted a survival analysis (allowing multiple failures per herd) to compare the hazard of having a BTB episode in any of the four groups of exposed herds vs. the hazard in herds in the reference group. We controlled for other known risk factors as well taking into account a temporal component. Our analysis showed that the hazard ratio for the index herds (group 1) were non-significantly

increased, indicating that there was no difference in the hazard of failing a BTB test (after the targeted badger removal was conducted) between index herds and reference herds. For the rest of the herds farther away from badger removal activities the hazards were lower than herds in areas not under badger removal. The hazard in the reference group decreased over the study period.

This article was published in Preventive Veterinary Medicine, 88, Olea-Popelka, F.P., Fitzgerald, P., White, P., McGrath, G., Collins, J.D., O'Keeffe, J., Kelton, D.F., Berke, O., More, S.J., Martin S.W., Targeted badger removal and the subsequent risk of bovine tuberculosis in cattle herds in county Laois, Ireland, 178-184, Copyright Elsevier B.V. 2009.

The impact of targeted badger removal on tuberculosis in cattle herds in Co. Monaghan

White, P.^{1, 2}, Frankena, K.³, O'Keeffe, J.^{1, 2}, More, S.J.², de Jong, M.³, Martin, S.W.⁴

¹ Department of Agriculture, Fisheries and Food, ² UCD CVERA, ³ Wageningen Institute of Animal Sciences, Wageningen University, The Netherlands,

⁴ Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada

Following the establishment of the Wildlife Unit in October 2003, a policy of targeted badger removal in the areas surrounding TB breakdown herds was implemented in the Republic of Ireland. This study will assess the impact of targeted badger removal on the subsequent levels of TB in herds in Co. Monaghan.

An assessment of injury to European badgers (*Meles meles*) due to capture in stopped restraints

Murphy, D.¹, O'Keeffe, J.J.^{2, 3}, Martin, S.W.⁴, Gormley, E.¹, Corner, L.A.L.¹

¹ UCD TB Diagnostics and Immunology Research Centre, ² Department of Agriculture, Fisheries and Food, ³ UCD CVERA,

⁴ Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada

Journal of Wildlife Diseases 45, 481-490 (2009)

As part of ongoing culling operations, European badgers (*Meles meles*) were captured using stopped restraints in winter (October to December 2005) and summer (May to June 2006) in the Republic of Ireland. A subset of these badgers, those caught during four consecutive nights, was examined postmortem to determine the frequency and severity of physical injuries resulting from capture in the restraints. The skin and the tissues underlying the restraint of 343 badgers were assessed for injury by visual examination. There was an absence of skin damage or only minor skin abrasions in 88% of badgers; an absence of subcutaneous tissue injury or only localized subcutaneous tissue injury in 69%; and an absence of muscle injury or only slight muscle bruising in 99% of badgers. Only 2% of badgers had cuts to the skin and 5.5% had extensive subcutaneous edema, whereas 1.2% had areas of hemorrhage and tearing of the underlying muscle. Our results show that the majority of badgers examined sustained minimal injuries attributable to capture in stopped restraints.

Printed with permission from the Wildlife Disease Association.

Progress towards a badger vaccine

Badger tuberculosis vaccine

Corner, L.A.L.¹, Murphy, D.¹, Costello, E.², Gormley, E.¹

¹ UCD TB Diagnostics and Immunology Research Centre, ² Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food

The research on BCG vaccination against tuberculosis in captive badgers, an integrated series of experiments and associated studies is continuing, and a series of studies have been completed that has led to commencement of field vaccination trial of wild badgers. The BCG vaccine was initially chosen for use based on its availability, low production cost and much experience of its application in domestic and wild animals, and humans. To carry out these studies in a controlled environment, the Badger Research and Observation Centre (BROC) was built and designed to hold 6-7 small groups of badgers. In parallel with the captive badger experiments, studies have also been undertaken in wild, naturally infected badgers, using badgers removed during culling operations. As part of these studies, we helped develop and assess a range of *in vitro* diagnostic assays based on the immunological responses to challenge with virulent *M. bovis*. In our studies carried out at the BROC, we have shown that the BCG vaccine is protective in badgers by the subcutaneous and mucosal routes, and by the oral route. The problem of the potential destruction of BCG by stomach acids as it passes through the GI tract has been addressed by the development of the lipid-base encapsulation matrix that is able to resist degradation by gastric enzymes (Dr. Frank Aldwell, Otago University, New Zealand).

BROC 1: Experimental infection with M. bovis

As a first step in development of a vaccine, an infection model was required to generate disease in captive badgers. The objective of BROC 1 was to identify a dose of *M. bovis* which, when delivered by the endobronchial route to badgers, generated a disease profile that mimicked natural disease. The results showed that over a wide range of doses, the endobronchial procedure produced disease that was characteristic of natural disease.

BROC 2: Progression of the experimental infection

Having established the utility of the endobronchial route and an effective challenge dose, this study was designed to use the optimal infective dose (derived from BROC 1) and follow the progression of the disease over time. This was important to determine the optimal time to examine the differences in the progression of disease between vaccinates and controls. Following experimental challenge, infection progressed slowly with a uniform result across the badgers studied. The optimal time to examine disease in vaccinates was found to be 12 to 18 weeks after infection.

BROC 3: Establishing proof that BCG induces protection against tuberculosis in badgers

With the infection procedure established, we set out to determine if BCG was protective in badgers and to examine possible routes of vaccination including the subcutaneous and mucosal routes. The results showed that vaccination by either route led to significant protection of vaccinated badgers compared with non-vaccinated controls.

BROC 4: Oral BCG vaccination and protection

To be of practical use in the field delivery of BCG, an oral bait is likely to be the most cost-effective means of delivery. A lipid formulation that protects the live BCG from gastric secretions has been developed by Dr Frank Aldwell (University of Otago, New Zealand). Having demonstrated that BCG generated protection in badgers, and that a mucosal route was highly effective, we wanted to test the efficacy of BCG vaccine delivered by the oral route after challenge by the endobronchial infection procedure. The outcome was that vaccination by the oral route led to significant protection.

BROC 5: Duration of protection following oral BCG vaccination

Having demonstrated that oral delivered BCG could induce protection, we set out to determine if badgers vaccinated by oral routes with a lipid-encapsulated BCG would induce protection that could be detected for up to 12 months. The study is due for completion.

BROC 6: Comparison of protection of badgers vaccinated with BCG-Pasteur and commercial BCG-Danish vaccine strains

To date, all of our studies have been conducted using the BCG-Pasteur strain. However, currently the only BCG vaccine strain produced and registered in the EU is the BCG-Danish strain manufactured by Statens Serum Institut (Denmark). There is now an international consensus that the vaccine submitted for registration as veterinary medicine will use this strain. In this study, we compared oral vaccination with the two BCG strains by feeding badgers with the BCG strains encapsulated in a semi-solid lipid matrix that was prepared specifically for this purpose by the collaborating laboratory in New Zealand (Dr Frank Aldwell, University of Otago). The results indicate that both vaccines generated high levels of protective immunity. There are no significant differences between BCG-Danish and BCG-Pasteur strains.

*BROC 7: Protective efficacy of BCG vaccination against a low dose challenge with *M. bovis**

In the wild, under conditions of natural transmission, badgers are exposed to and can become infected with low doses of *M. bovis*. In our initial BROC experimental infection studies we demonstrated that it was possible to infect a badger with



as low <10 colony forming units (CFU) of *M. bovis*. The purpose of the BROC 7 study was to determine what effect vaccination would have on the experimental disease following a low challenge dose and over a longer timescale. Badgers were vaccinated with BCG-Danish strain encapsulated in a semi-solid lipid matrix. This allows for the vaccine to be delivered by the oral route and the matrix protects the vaccine from being degraded in the stomach. A control group remained non-vaccinated. Twelve weeks after vaccination, the badgers were challenged by the endobronchial route with a low dose of *M. bovis* (3×10^2 CFU). At 52 weeks post-challenge the badgers were euthanased and protection assessed by pathology and culture. As measured by distribution and severity of lesions there was significant immune protection generated in the vaccinated group of badgers. The data has given us a much better understanding of how the vaccine is likely to perform under natural conditions. This will facilitate the development of strategies to deliver the vaccine to badger populations.

Delivery of the oral vaccine. Photography by the Badger Vaccine Project.

Immunological responses and protective immunity in BCG vaccinated badgers following endobronchial infection with *Mycobacterium bovis*

Lesellier, S.¹, Corner, L.A.L.¹, Costello, E.², Lyashchenko, K.³, Greenwald, R.³, Esfandiari, J.³, Singh, M.⁴, Hewinson, R.G.⁵, Chambers, M.⁵, Gormley, E.¹

¹ UCD TB Diagnostics and Immunology Research Centre, ² Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food,

³ Chembio Diagnostic Systems, Inc., New York, USA, ⁴ LIONEX GmbH, Braunschweig, Germany,

⁵ Veterinary Laboratories Agency, Weybridge, Surrey, England

Vaccine 27, 402-409 (2009)

European badgers (*Meles meles*) are a reservoir host of *Mycobacterium bovis* and are implicated in the transmission of tuberculosis to cattle in Ireland and Great Britain. The development of a vaccine for use in badgers is considered a key element of any campaign to eradicate the disease in livestock in both countries. In this study we have vaccinated groups of badgers with approximately 5×10^5 cfu of the BCG vaccine delivered via two alternative routes, subcutaneous and mucosal (intranasal/conjunctival). Following experimental endobronchial infection with approximately 10^4 cfu of *M. bovis*, all badgers were euthanised at 12 weeks post-infection. At post-mortem examination both vaccinated groups had significantly reduced severity of disease compared with the non-vaccinated controls. The analysis of immune responses throughout the study showed that vaccination with BCG did not generate any detectable immunological responses as measured by IFN-gamma production in antigen-stimulated peripheral blood mononuclear cells (PBMC) and IgG serological responses. However, the levels of the responses increased following *M. bovis* infection, and the kinetic profiles corresponded to the severity of lesions recorded post-mortem. Significant differences were observed in the timing of development of the immune responses between vaccinates and controls. The results suggest that the immunological responses are associated with the levels of protective immunity and could be used as markers to monitor control of disease in badgers following vaccination.

This article was published in Vaccine, 27, Lesellier, S., Corner, L.A.L., Costello, E., Lyashchenko, K., Greenwald, R., Esfandiari, J., Singh, M., Hewinson, R.G., Chambers, M., Gormley, E., Immunological responses and protective immunity in BCG vaccinated badgers following endobronchial infection with Mycobacterium bovis, 402-409, Copyright Elsevier Ltd. 2009.

Immunological responses following experimental endobronchial infection of badgers (*Meles meles*) with different doses of *Mycobacterium bovis*

Lesellier, S.¹, Corner, L.A.L.¹, Costello, E.², Sleeman, D.P.³, Lyashchenko, K.P.⁴, Greenwald, R.⁴, Esfandiari, J.⁴, Hewinson, R.G.⁵, Chambers, M.⁵, Gormley, E.¹

¹ UCD TB Diagnostics and Immunology Research Centre, ² Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food,

³ Department of Zoology, Ecology and Plant Science, University College Cork, ⁴ Chembio Diagnostic Systems, Inc., New York, USA,

⁵ Veterinary Laboratories Agency, Weybridge, Surrey, England

Veterinary Immunology and Immunopathology 127, (2009) 174-180

The Eurasian badger (*Meles meles*) is a wildlife reservoir for *Mycobacterium bovis* infection in Ireland and Great Britain and has been implicated in the transmission of tuberculosis to cattle. Vaccination of badgers is an option that could be used as part of a strategy to control the disease. In this study we used an endobronchial infection procedure to inoculate groups of badgers with three different doses (3×10^3 , 2×10^2 and <10 Colony Forming Units (CFUs)) of *M. bovis*. After 17 weeks the disease status of each animal was determined by post-mortem pathology and culture for *M. bovis*. Each of the inoculum doses resulted in establishment of infection in the badgers. The cell-mediated immune (CMI) responses were measured by lymphocyte transformation assay (LTA) of peripheral blood mononuclear cells (PBMCs) cultured with

bovine tuberculin (PPD-B). In each infected group the CMI responses increased with a kinetic profile corresponding to the delivered dose and the post-mortem pathology. The serological responses were measured by ELISA and a multi-antigen print immunoassay (MAPIA) in order to investigate any changes in the antigenic repertoire associated with different infective doses. In contrast to the CMI responses, the ELISA and MAPIA showed that the recognition of antigens by the badgers was intermittent and not strongly influenced by the dose of *M. bovis*.

This article was published in Veterinary Immunology and Immunopathology, 127, Lesellier, S., Corner, L.A.L., Costello, E., Sleeman, D.P., Lyashchenko, K.P., Greenwald, R., Esfandiari, J., Hewinson, R.G., Chambers, M., Gormley, E., Immunological responses following experimental endobronchial infection of badgers (Meles meles) with different doses of Mycobacterium bovis, 174-180, Copyright Elsevier B.V 2009.

Vaccination of European badgers (*Meles meles*) with BCG by the subcutaneous and mucosal routes induces protective immunity against endobronchial challenge with *Mycobacterium bovis*

Corner, L.A.L.¹, Costello, E.², Lesellier, S.¹, O'Meara, D.², Gormley, E.¹

¹ UCD TB Diagnostics and Immunology Research Centre, ² Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food

Tuberculosis 88, 601-609 (2008)

Mycobacterium bovis is endemic in badger (*Meles meles*) populations of Ireland and the United Kingdom and infected badgers are a potential source of infection for cattle. In domestic livestock tuberculosis causes economic losses from lost production and the costs associated with eradication programmes, and in addition there is a risk of zoonotic infection. Whereas culling is currently used to control tuberculous badger populations in Ireland, vaccination, if it were available, would be preferred. A study was undertaken to examine the protective responses of badgers vaccinated either by the subcutaneous or mucosal (intranasal and conjunctival) routes with bacille Calmette-Guérin (BCG), when challenged with *M. bovis* by the endobronchial route. Three groups of badgers were used. The first group (n=4) was vaccinated with approximately 5×10^5 colony forming units (cfu) of BCG by subcutaneous injection. In the second group (n=5) badgers were vaccinated via the mucosal route by instilling 1.0×10^5 cfu into each conjunctival sac and spraying 1.0×10^5 cfu into each nostril (final vaccine dose of 4×10^5 cfu). The control (n=5) badgers served as a non-vaccinated group. Twelve weeks post-vaccination all badgers in the three groups were challenged with approximately 10^4 cfu of *M. bovis* by endobronchial inoculation. At 12 weeks post-infection all badgers were examined post-mortem to assess the pathological and bacteriological responses to challenge. Gross and histological lesions of tuberculosis were seen in all challenged badgers and *M. bovis* was recovered from all challenged badgers. However, across six of the eight parameters used to measure disease severity, the infection in the vaccinated badgers was significantly less severe than in the control group. The BCG vaccine induced a significant protective effect in the badgers and the protective immunity was generated by subcutaneous and mucosal vaccination.

This article was published in Tuberculosis, 88, Corner, L.A.L., Costello, E., Lesellier, S., O'Meara, D., Gormley, E., Vaccination of European badgers (Meles meles) with BCG by the subcutaneous and mucosal routes induces protective immunity against endobronchial challenge with Mycobacterium bovis, 601-609, Copyright Elsevier Ltd. 2008.

Experimental tuberculosis in the European badger (*Meles meles*) after endobronchial inoculation with *Mycobacterium bovis*: II. Progression of infection

Corner, L.A.L.¹, Costello, E.², Lesellier, S.¹, O'Meara, D.¹, Gormley, E.¹

¹ UCD TB Diagnostics and Immunology Research Centre, ² Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food

Research in Veterinary Science 85, 481- 490 (2008)

The aim of the study was to describe, over a period of 24 weeks, the pathological and bacteriological changes in badgers experimentally infected with *Mycobacterium bovis*. The badgers were infected by endobronchial instillation of 2.5×10^4 colony forming units (cfu) *M. bovis*. After infection, the badgers were examined at 3 weekly intervals when blood and tracheal aspirates were collected. At 6, 12, 18 and 24 weeks post-infection (pi) three animals were euthanized and a detailed pathological and bacteriological examination was performed to assess the nature of the experimental disease. During the course of the study only one badger developed clinical signs of disease: a subcutaneous swelling on its head, first observed at 18 weeks pi. At post-mortem examination gross and histological lesions of tuberculosis were observed and *M. bovis* was recovered from all, except one badger. In the majority of badgers the endobronchial route of inoculation resulted in the establishment of infection that over 24 weeks was non-progressive with limited dissemination of infection from the thoracic cavity, mainly to the hepatic and mesenteric lymph nodes. However, in one of the badgers examined at 18 weeks pi and one at 24 weeks pi, infection was widely disseminated. The disease induced by the endobronchial inoculation displayed the characteristics of disease observed in naturally infected badgers.

This article was published in Research in Veterinary Science, 85, Corner, L.A.L., Costello, E., Lesellier, S., O'Meara, D., Gormley, E., Experimental tuberculosis in the European badger (Meles meles) after endobronchial inoculation with Mycobacterium bovis: II. Progression of infection, 481-490, Copyright Elsevier Ltd. 2008.



Antigen specific immunological responses of badgers (*Meles meles*) experimentally infected with *Mycobacterium bovis*

Lesellier, S.^{1, 6}, Corner, L.A.L.¹, Costello, E.², Sleeman, D.P.³, Lyashchenko, K.⁴, Greenwald, R.⁴, Esfandiari, J.⁴, Singh, M.⁵, Hewinson, R.G.⁶, Chambers, M.⁶, Gormley, E.¹

¹ UCD TB Diagnostics and Immunology Research Centre, ² Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food,

³ Department of Zoology, Ecology and Plant Science, University College Cork, ⁴ ChemBio Diagnostic Systems, Inc., New York, USA, ⁵ Helmholtz Center for Infectious Research; and LIONEX GmbH, Braunschweig, Germany, ⁶ Veterinary Laboratories Agency, Weybridge, Surrey, England

Veterinary Immunology and Immunopathology 122, 35-45 (2008)

European badgers (*Meles meles*) are considered to be an important reservoir of infection for *Mycobacterium bovis* and are implicated in the transmission of tuberculosis to cattle in Ireland and Great Britain. Accurate tests are required for tuberculosis surveillance in badger populations and to provide a basis for the development of strategies, including vaccination, to reduce the incidence of the infection. In this study, we have developed an endobronchial *M. bovis* infection model in badgers in which we measured cell-mediated immune and serological responses for up to 24 weeks post-infection. Groups of badgers were subjected to necropsy at 6-week intervals and the gross lesion severity status compared with immune responses measured in blood samples taken throughout the course of the study. The panel of antigens included bovine and avian tuberculins (PPD) as well as single antigens, ESAT-6, CFP-10, MPB70, Rv3019c, Rv3873, Rv3878 and Rv3879, all known to be recognised by the immune system in other animal models of tuberculosis infection. Our results demonstrated that *M. bovis* infected badgers responded to specific antigens as early as 6 weeks post-infection, consistent with the presence of visible lesions. The data also revealed unique patterns of antigen recognition with high levels of PBMC proliferation in the presence of CFP-10 but low proliferation levels with ESAT-6. Using a multi-antigen print immunoassay (MAPIA), we were able to confirm that MPB83 is the dominant antigen recognised by serum antibodies in infected badgers.

This article was published in Veterinary Immunology and Immunopathology, 122, Lesellier, S., , Corner, L.A.L., Costello, E., Sleeman, D.P., Lyashchenko, K., Greenwald, R., Esfandiari, J., Singh, M., Hewinson, R.G., Chambers, M., Gormley, E., Antigen specific immunological responses of badgers (Meles meles) experimentally infected with Mycobacterium bovis, 35-45, Copyright Elsevier B.V. 2008.

The vaccine field trial

Whereas captive badger studies are the most cost effective way of examining the protective response to vaccination, such studies cannot be used to predict whether BCG will be protective in free-ranging badgers or to estimate vaccine efficacy. Estimates of vaccine efficacy are extremely valuable in modelling potential vaccine strategies, but data from field trials are needed to reliably estimate protection and vaccine efficacy parameters. Any field trial will by necessity use an oral vaccine delivery system, because this is the likely method of choice for any broad-scale mass vaccination of free-ranging badger populations.

The BCG vaccine field trial has two principal objectives. These are to validate the results of captive badger studies and show that BCG vaccine is protective in naturally exposed wild badgers, and to estimate vaccine efficacy under field conditions. These objectives will be met by comparing the prevalence of *M. bovis* infection in vaccinated badgers with that in non-vaccinated controls. A secondary outcome of field trials will be to measure the effect of BCG vaccine in badgers with pre-existing *M. bovis* infection. In addition to providing a measurement of protection and an estimate of vaccine efficacy, the field trial will provide a practical basis for understanding the logistics of oral vaccine delivery to wild badger populations.

In the field trial area in Co. Kilkenny, different proportions (0, 50, and 100%) of the badger population are being vaccinated with BCG or placebo. The advantage of this design is that effects on vaccine efficacy arising from changes in the force of infection as a result of different levels of vaccination coverage can be estimated. The required proportion of vaccinates is achieved by systematically trapping the area. When first encountered, individual badgers are allocated to either the vaccination or control group as required for the particular area. To allow for continued exposure to infection, the trial will be conducted over a 3-yr period. It is estimated that an initial population of 300 badgers (100 in each of the treatment areas) will be required to accurately estimate vaccine efficacy, based on an assumed initial tuberculosis prevalence of 20–30% and vaccine efficacy of 50–70% for an individual badger.

The BCG Danish strain, encapsulated in a lipid formulation for oral administration and containing about 10^8 cfu/ml, is being used in the field trial. Badgers are individually vaccinated by administration of the lipid vaccine or lipid placebo directly into the pharynx. Vaccine and placebo control samples are “double-blind” coded. Badgers will be revaccinated annually and the population examined two times per year by trapping the entire study site in a continuous process. Throughout the trial, estimates of changing tuberculosis incidence will be made from the measurements of humoral immune responses.

At the end of the 3-yr study period, the trial site will be depopulated and all badgers will be examined for tuberculosis by detailed postmortem examination that will include an examination for visible lesions, histologic lesions, and mycobacteriology to demonstrate infection with *M. bovis*. The isolation of *M. bovis* from post-mortem or clinical samples (wound exudates or tracheal swabs) will be used to define a case of tuberculosis. The results and experience gained from the field trial will facilitate the development of strategies for introduction of vaccination into the national program. The trial commenced in 2009.

Trial design to estimate the effect of vaccination on tuberculosis incidence in badgers

Aznar, I.¹, McGrath, G.¹, Murphy, D.², Corner, L.A.L.², Gormley, E.², Frankena, K.³, More, S.J.¹, Martin, S.W.⁴, O’Keeffe, J.^{1, 5}, de Jong, M.³

¹ UCD CVERA, ² UCD TB Diagnostics and Immunology Research Centre, ³ Wageningen Institute of Animal Sciences, Wageningen University, The Netherlands,

⁴ Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada, ⁵ Department of Agriculture, Fisheries and Food

The principal wildlife reservoir of *Mycobacterium bovis* in Ireland is the European badger. Studies in the Republic of Ireland (RoI) have shown that badgers culled in association with cattle herd tuberculosis (TB) breakdowns (focal culling) have a higher prevalence of infection than the badger population at large. This observation is one rationale for the medium term national strategy of focal badger culling. A vaccination strategy for the control of TB in badgers is a preferred long-term option. The Bacillus Calmette-Guérin (BCG) vaccine has been shown to decrease disease severity in captive badgers under controlled conditions. As the vaccine has been tested in a controlled environment with precise information on infection pressure, it cannot be assumed *a priori* that the effects of vaccination are similar in the wild, where other environmental and/or ecological factors prevail. For this reason we have designed a vaccine field trial to assess the impact of vaccination on the incidence of TB infection in a wild badger population. The selected study area for the vaccine trial (approximately 755 square kilometers) is divided into three zones each of which has similar characteristics in terms of size, number of main badger setts, cattle herds, cattle and land classification type. Three vaccination levels (100%, 50% and 0%) will be allocated to the three zones in a way that a gradient of vaccination coverage North to South is achieved. The middle zone (Zone B) will be vaccinated at a 50% coverage but Zone A and C will be randomly allocated with 100% or 0% vaccination coverage. Vaccination within Zone B will be done randomly at individual badger level. The objective of this paper is to describe the trial design, the epidemiological methods that were used to design the trial and the subsequent data analysis. The analysis will enable evaluation of the effect of vaccination on disease transmission under field conditions. It also aims to quantify the magnitude of the observed vaccination effect on transmission and to improve our knowledge on the biological effects of the vaccination on susceptibility and infectiousness of badgers.

The development of a bait delivery system for use in oral vaccination of badgers against bovine tuberculosis

Kelly, D.¹, Corner, L.A.L.², Gormley, E.², Marples, N.M.¹

¹ School of Natural Sciences, Trinity College Dublin, ² UCD TB Diagnostics and Immunology Research Centre

This project aims to formulate a protocol for the efficient delivery of BCG vaccine to badgers in order to reduce the prevalence of bovine tuberculosis in wildlife. Working initially with captive animals at the DAFF BROCC facility, a number of candidate oral vaccine bait additives were tested as attractants. The captive study demonstrated that carob and cocoa were more attractive to captive badgers than a peanut flavour. The captive animals were required to overturn a floor tile (approximately 2 kg in weight) to gain access to the experimental baits. This proved to be a simple task if they were attracted to the bait. This work will shortly be submitted to the *European Journal of Wildlife Research*.

Some preliminary studies with a wild sett near to the BROCC facility showed that the carob powder was a successful attractant for wild badgers. Floor tiles were used to prevent bait access from rodents or birds (as in the captive studies). These preliminary studies also showed that wild animals could learn to remove candidate oral vaccine baits from a simple wax-coated paper package. The ultimate deployment strategy for the oral vaccine bait requires the baits to be packaged. This important finding also informed the design of a wild trial.

In the wild trial, animals were offered a choice of three packaged baits (peanut, carob and cocoa flavours). The baits were buried in 15cm-deep holes adjacent to main sett entrances, and the holes backfilled with earth. The disappearance

of baits from these holes proved that the wild badgers could detect and excavate baits from deep backfilled holes. The results showed that peanut flavour was a more popular attractant than both carob and cocoa butter for wild badgers. No baits were removed from their holes on the first day of presentation, indicating that a pre-baiting period of at least one day would be necessary for live vaccine bait deployment. The wild badgers quickly learned to associate all attractants with the prototype baits and continued to remove them from buried sites, even when the attractants were absent. This suggests that it may be possible to train badgers to feed at a site convenient for the personnel deploying the vaccine baits. A manuscript describing this work is in preparation.

A protocol for a training trial with wild animals, in Wicklow, has been agreed. Fieldwork should be underway by the end of February 2010. It will test whether badgers that are unfamiliar with packaged baits can learn to extract them without additional attractant cues. This may ultimately save money, as the use of attractants on each packaged bait would increase manufacturing costs.

In addition to bait studies, it has been possible to investigate the feeding behaviour of the captive animals at BROCC using stable isotope analysis. This has provided a fundamental baseline value for Eurasian badgers which may open the door for more detailed trophic studies both in Ireland and the UK. Additional data collected from animals during their 'winter lethargy' may go some way to understanding how they utilise their stored adipose supplies during the winter months.

Control of TB in wildlife by oral BCG vaccination

Gormley, E.¹, Corner, L.A.L.¹

¹ UCD TB Diagnostics and Immunology Research Centre

Expert Review of Vaccines 8, 1339-1342 (2009)

Tuberculosis caused by *Mycobacterium bovis* is present in many wild animal populations throughout the world. The disease can have a significant economic impact when the wild animal species is a reservoir of infection for domestic animals. It can pose a zoonotic threat for those who come in contact with infected animals, be they wild or domesticated. To date, strategies for dealing with TB in wildlife have been limited; physical separation of wild from domestic animals or culling of infected populations can help to reduce the spread of infection, but culling is not an option with species of high conservation value. Vaccination of animals with the bacillus Calmette-Guérin (BCG) vaccine has long been considered an attractive strategy for combating the disease. However, its widespread use in the wild has been constrained pending the development of a suitable delivery system. In the paper under evaluation, Tompkins and colleagues in New Zealand have shown that oral vaccination with BCG can protect wild brushtail possums against natural infection with *M. bovis*. This highly significant finding paves the way towards incorporation of wildlife vaccination into bovine TB eradication programs worldwide.

Printed with permission from Expert Reviews Ltd.

Evaluation of badger group territories in four Irish counties by means of colour-marked baits

Smal, C.M.¹, Brown, J.A.¹

¹ Independent consultants

Badger territorial activity was investigated (in 2002) by a bait-marking technique employed at five study areas located within the Reference Areas of the Four Area Project (Co. Cork, Donegal, Kilkenny and Monaghan). The methodology involved feeding coloured marker pellets, contained within an attractive food mix, to badgers and locating the subsequent dispersal of the defecated pellets. Badgers are social animals that defend their territories both physically and by means of scent marking and defecation sites (such latrine sites are often located along the boundaries of their territories).

The areas selected for study varied in size from c. 5km² in Co. Donegal to c. 16km² in Co. Kilkenny. 40 setts were fed with marked bait and 34 social groups were identified by evaluation of pellet returns. 1054 latrine sites were identified, with 547 containing marker pellets. The interpreted territory size of the 34 groups varied between 15ha and over 200ha, with a mean of 80ha. Mean badger group density was estimated at 0.8 social groups per km². The study area chosen in Co. Donegal was found to possess a very high badger density (c. 1.4 groups per km²), whereas densities in other counties were closer to expectation.

The badger groups studied displayed relatively well-defined territorial boundaries, typical of those known from other significant studies in Ireland and Britain. There were found examples, in each county, of long-range movements - including movements of individual badgers between main setts and also between territories. Individual badgers may not range over all of the group's territory and it is likely that some badgers are not always present at a group's main sett. These observations have implications for sett survey and also for proposed targeting of badgers with vaccines incorporated into baits.

The study confirmed that each badger group has, almost without exception, one identifiable active main sett within its territory. Field survey of badger setts in any given area may, therefore, be conducted by experienced personnel with enumeration of active main setts and with expectation of reliable assessment of badger group density.

Tuberculosis in European badgers (*Meles meles*) and the control of infection with bacillus Calmette-Guérin vaccination

Corner, L.A.L.¹, Murphy, D.¹, Costello, E.², Gormley, E.¹

¹ UCD TB Diagnostics and Immunology Research Centre, ² Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food

Journal of Wildlife Diseases 45, 1042-1047 (2009)

The eradication of tuberculosis (*Mycobacterium bovis* infection) from cattle herds may be compromised if infected wild-life species, such as European badgers (*Meles meles*), share the same environment and contribute to transfer of infection. Options for dealing with tuberculosis in this wild reservoir host are limited by conservation and social concerns, despite a clear implication that infected badgers are involved with the initiation of tuberculosis in cattle herds. Vaccination of badgers against *M. bovis*, if successfully employed, would directly facilitate the completion of bovine tuberculosis eradication in affected areas. Vaccine trials in captive badgers have established that the *M. bovis* bacille Calmette-Guérin (BCG) vaccine can induce a protective response that limits the distribution and severity of tuberculosis disease following experimental challenge. The protective effect of the vaccine has been demonstrated when the vaccine was delivered by subcutaneous injection, deposited on mucous membranes, and given orally in a lipid formulation. A large-scale field trial of oral BCG vaccine has been designed to measure the protection generated in wild badgers subjected to natural trans-

mission of infection and to estimate vaccine efficacy. These parameters will be estimated by comparing the prevalence of *M. bovis* infection in vaccinated and non-vaccinated badgers. The results will provide a framework for the development and implementation of a national strategy to eliminate the disease in badger populations and if successful will remove this major impediment to bovine tuberculosis eradication.

Printed with permission from the Wildlife Disease Association.

Supporting studies

Badger immunodiagnostics

Development of badger immunodiagnostics

In tandem with the badger vaccine research, we have been developing and assessing a range of immunodiagnostic tests that will be required for tuberculosis surveillance in badger populations to monitor the effect of vaccination. This work has been carried out in collaboration with VLA Weybridge. More recently we have been working with Enfer group on developing antibody based diagnostic tests for badgers. This has the advantage of using serum samples, which can be easily stored prior to testing and removes the impediment for immediate transport of blood samples to the laboratory for testing.

Sensitivity of immunological assays for diagnosis of tuberculosis in wild badgers

A range of serological and cell mediated immunity (CMI) based assays have been developed recently and have shown encouraging results when used in captive badgers. However, there is a need to validate these tests on naturally infected badgers. The aim of the current study was to evaluate five of these diagnostic immunoassays (the Brock test, the BrockTB STAT-PAK®, the lymphocyte transformation assay (LTA), the interferon-gamma (IFN- γ) ELISA and the ELISPOT assay) in wild badgers by comparing test results to the diagnostic gold standard, i.e. bacterial culture using a comprehensive range of lymph nodes and tissues collected from badgers. Blood samples were collected from culled badgers (n = 215) prior to euthanasia and all badgers were examined *post mortem* with bacterial culture of tissues. Based on ROC analysis, the CMI assays had higher sensitivities than the serological assays in this study, the LTA having the highest sensitivity (54.9%, apparent specificity 86.6%) and the BrockTB STATPAK the lowest sensitivity (30.8%, apparent specificity 97.8%). The sensitivity of all the immunodiagnostic assays improved as the disease severity increased and this was more pronounced with the serological based assays than the CMI assays. Overall, the individual immunodiagnostic assays assessed in this study had low sensitivities for the accurate diagnosis of *M. bovis* infection in badgers when compared to the gold standard of bacteriological culture. However, the combination of two or more assays improved the diagnostic accuracy of the tests.

Adverse reactions to vaccine usage

Adverse reactions to *Mycobacterium bovis* bacille Calmette–Guérin (BCG) vaccination against tuberculosis in humans, veterinary animals and wildlife species

Murphy, D.¹, Corner, L.A.L.¹, Gormley, E.¹

¹ UCD TB Diagnostics and Immunology Research Centre

Tuberculosis 88, 344-357 (2008)

The *Mycobacterium bovis* strain, bacille Calmette–Guérin (BCG) is one of the most widely used human vaccines and remains one of the safest vaccines available. It has been used in human populations for over 80 years and 100 million children receive the vaccine annually. It has also been employed extensively for vaccine studies in laboratory animal hosts and is currently being developed for use in a variety of livestock and wild animals. Despite the large number of doses delivered since its first usage in 1921, reports of adverse reactions arising from the use of the BCG vaccine are relatively uncommon and where serious reactions do occur they are often the result of vaccination of immuno-compromised individuals. Factors that may influence the development of adverse reactions to BCG include the potency and dose of the vaccine strain, the route of delivery, the age and immune status of the host, and the skill levels of the operator administering the vaccine. Circumstances affecting the notification of adverse reactions include the lack of clear case definitions of abnormal vaccine reactions, and a scarcity of systematic surveillance and functioning reporting systems. With continued use of the BCG and the development of a new generation of prophylactic and therapeutic vaccines against tuberculosis in different host species, the risk factors associated with adverse reactions may need to be reappraised.

This article was published in Tuberculosis, 88, Murphy, D., Corner, L.A.L., Gormley, E., Adverse reactions to Mycobacterium bovis bacille Calmette–Guérin (BCG) vaccination against tuberculosis in humans, veterinary animals and wildlife species, 344-357, Copyright Elsevier Ltd. 2008.

Mathematical modelling

Infection dynamics and effective control strategies of tuberculosis in badgers and cattle of Ireland

Aznar, I.¹, McGrath, G.¹, Corner, L.A.L.², Gormley, E.², Frankena, K.³, More, S.J.¹, Martin, S.W.⁴, O’Keeffe, J.^{1, 5}, de Jong, M.³

¹ UCD CVERA, ² UCD TB Diagnostics and Immunology Research Centre, ³ Wageningen Institute of Animal Sciences, Wageningen University, The Netherlands,

⁴ Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada, ⁵ Department of Agriculture, Fisheries and Food

Recent research indicates that bovine tuberculosis (bTB, infection with *Mycobacterium bovis*) is endemic in the badger population in Ireland, and that disease in badgers contributes to the problem in cattle. Despite efforts to reduce cattle-to-cattle transmission through a comprehensive national surveillance program and the reactive badger culling policy, bTB has remained at a relatively constant, albeit low, prevalence in the cattle population of Ireland. It is thought that a strategy involving vaccination of badgers, possibly in combination with other intervention strategies, will help to reduce the incidence of infection in badgers. Modelling various scenarios involving a number of different interventions will help determine the impact that these interventions may have on the bTB prevalence in both cattle and badger, and will help to inform further research and policy decisions. The main objective is to assess the impact of interventions on bovine tuberculosis (bTB) prevalence in cattle and badgers; for this a mathematical model of bTB transmission that describes the disease in cattle and badgers in the Republic of Ireland is being developed. A conceptual framework of how bTB persists in both badgers and cattle has been constructed, using mathematical modelling. The next step of the project will

involve the use of existing data to get quantitative estimates of the required model parameters. Irish data on cattle to cattle transmission have been analysed and the transmission parameter for cattle (β) has been estimated. The estimation of the inter-species transmission parameter is in progress.

Badger anaesthesia, clinical pathology

Bronchoalveolar lavage cytology from captive badgers

McCarthy, G.¹, Shiel, R.¹, O'Rourke, L.¹, Murphy, D.², Corner, L.A.L.², Costello, E.³, Gormley, E.²

¹ UCD School of Agriculture, Food Science and Veterinary Medicine, ² UCD TB Diagnostics and Immunology Research Centre,

³ Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food

Veterinary Clinical Pathology 38, 381-387 (2009)

Bronchoalveolar lavage (BAL) fluid is evaluated for the diagnosis and study of lung disease and airway inflammation. Cytologic profiles for BAL fluid have not been reported for badgers and may be useful in understanding the pathogenesis of pulmonary diseases such as *Mycobacterium bovis*. The aim of this study was to evaluate cytologic and microbial findings in BAL fluid from captive European badgers (*Meles meles*) and identify correlates with the results of concurrently collected blood and fecal samples. BAL fluid (by a nonbronchoscopic method) and jugular venous blood samples (for routine CBC) were obtained from 23 captive tuberculosis-free anesthetized badgers on 2 occasions 4 weeks apart. Fecal samples were collected for routine parasitology. Morphologic evaluation and 100-cell differentials were done on cytocentrifuged BAL specimens. Pellets from centrifuged BAL were aerobically cultured for bacteria. With the 2 BAL samples from each of the 23 badgers combined, the median (range) cell percentages were 73.0% (5–95%) neutrophils, 7.5% (2–16%) macrophages, 8.0% (0–27%) lymphocytes, and 9.5% (0–92%) eosinophils. Macrophages frequently contained silica-like crystals. Other findings included ciliated epithelial cells, goblet cells, mucus, and *Aelurostrongylus* sp. larvae. A light growth of *Streptococcus*, *Pasteurella*, or *Escherichia coli* was cultured in 6 badgers. *Trypanosoma pestanaei* were identified in blood from 10 badgers and fecal parasites (mainly coccidia) were found in 20 badgers. No correlation was found between BAL and CBC results and the presence of parasites. The predominance of neutrophils in BAL fluid from badgers differs from the predominance of macrophages found in BAL from other species. This difference may reflect the burrowing lifestyle or the unique immune response of badgers.

Printed with permission from John Wiley & Sons, Inc.

The original article can be found at <http://www3.interscience.wiley.com/journal/122295015/abstract>



The national programme

A review of TB research in Ireland

More, S.J.¹, Gormley, E.²

¹ UCD CVERA, ² UCD TB Diagnostics and Immunology Research Centre

Research on bovine tuberculosis (TB) has been conducted in Ireland over the last 20 years or so. Much of this work has focused on gaps in knowledge of disease epidemiology, a critical assessment of disease control strategies, constraints to eradication and an evaluation of alternative strategy options. As yet, no summary is available of this substantial body of peer-reviewed work. This review paper seeks to fill this gap.

What is needed to eradicate bovine tuberculosis successfully: an Irish perspective

More, S.J.¹

¹ UCD CVERA

The Veterinary Journal 180, 275-278 (2009)

The Irish national bovine tuberculosis (TB) eradication programme, first established in 1954, has proved effective in controlling TB caused by *Mycobacterium bovis*. However, eradication of the disease remains elusive. We now have a good understanding of the constraints to eradication, based on detailed research conducted in Ireland and elsewhere. But three critical issues will need to be resolved if Ireland is to be successful in moving from TB control to eradication:

- methods to sustainably prevent wildlife-to-cattle transmission;
- the introduction of measures for improved cattle controls, and
- a critical rethink of programme governance.

Any impact from the latter two requirements is likely to be severely limited prior to the introduction of the first, given that badgers can be considered an 'upstream' driver, with ongoing transmission of infection from badgers to cattle irrespective of current efforts to minimise cattle-to-cattle transmission. Each of these three issues are discussed in some detail in this 'Personal View' to *The Veterinary Journal*.

This article was published in The Veterinary Journal, 180, More, S.J., What is needed to eradicate bovine tuberculosis successfully: an Irish perspective, 275-278, Copyright Elsevier Ltd. 2009.

Quality control

The comparative performance of the single intradermal comparative tuberculin test in Irish cattle, using tuberculin PPD combinations from different manufacturers

Good, M.¹, Clegg, T.A.², Murphy, F.¹, More, S.J.²

¹ Department of Agriculture, Fisheries and Food, ² UCD CVERA

Ireland currently obtains its avian and bovine tuberculin purified protein derivatives (PPDs) from a single source. Because problems of supply or quality cannot be discounted, it is prudent that Ireland identify alternative supplier(s) as part of a broad risk management strategy. Therefore, the aim of this study was to compare the performance of a number of different tuberculin combinations (that is, pairings of bovine and avian PPD; with different manufacturers) in the single intradermal comparative tuberculin test (SICTT), as currently performed in Ireland. The study was randomised, controlled and double-blinded. A total of 2,172 cattle were used in the study. Each animal was tested using two SICTTs, the first based on the tuberculin combination in current use, and the second using one of six trial tuberculin combinations. Analyses were conducted to compare both reactor-status and skin increase. For each control/trial tuberculin combination, there was good agreement between the control and trial reactor-status. Differences in skin increases were mainly confined to animals categorised as either negative or severe inconclusive. However, the measured differences were minor, and unlikely to have a significant impact on the actual test outcome, either for individual animals or for herds. In conclusion, while further studies determining sensitivity and specificity in Ireland would have to be done in the event of a change in tuberculin PPD there should be minimal disruption of the national programme if alternative tuberculin PPDs of the same potency were used. In this study, the precision of the guinea pig bio-assay to assess tuberculin potency was low and therefore Ireland should maintain its practice of periodically assessing potency in naturally infected cattle.

The comparative performance of the single intradermal test and the single intradermal comparative tuberculin test in Irish cattle, using tuberculin PPD combinations of differing potencies

Good, M.¹, Clegg, T.A.², Costello, E.³, More S.J.⁴

¹ Department of Agriculture, Fisheries and Food, ² UCD CVERA, ³ Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food

Ireland currently obtains its avian and bovine tuberculin purified protein derivatives (PPDs) from a single source. Problems of supply or quality cannot be discounted. As yet, however, no work has been reported on the impact of tuberculins of different potencies on the performance of the single intradermal test (SIT) and the single intradermal comparative tuberculin test (SICTT). Therefore, the aim of this study was to compare the performance of bovine tuberculin PPDs of different potencies on the performance of the SICTT and SIT, as currently performed in Ireland and throughout Europe. The study was randomised, controlled and blinded. Three trial bovine tuberculins of varying potency, as assayed in naturally infected bovines, (low [1,192 IU/dose], normal [6,184], high [12,554]) were used in this study. Three SICTTs (based on the three trial tuberculins) were conducted on 2,102 animals. The results of these tests were compared, based on reactor-status and skin increases at the bovine site. There was a significant difference in the number of reactors detected using the high and low potency tuberculin. When used in a SICTT, the high potency and low potency tuberculins detected 40% more and 50% less reactors, respectively, than normal potency tuberculin. Further, the low potency tuberculin [X] failed to detect 20% of 35 animals with visible lesions. Tuberculin of different potencies will impact on reactor disclosure rates.

The development and application of new measures of performance

Higgins, I.¹, Williams, D.², More, S.J.¹

¹ UCD CVERA, ² UCD School of Mathematical Sciences

In recent years, there have been substantial advances in the analysis and management of the national bovine tuberculosis database in Ireland. The national Animal Health Computer System was upgraded in 2005, and facilitates the central collection and management of national data. This now provides over twenty years of data for research purposes. Furthermore, there has been a progressive improvement in methods at the Centre for Veterinary Epidemiology and Risk Analysis to manage and analyse this complex database, including the development of new episode-based perspectives in data analysis. With these advances, new statistical measures have been developed to assist with decision-making, both locally and at a national level. These measures, which relate to both programme activity and performance, are consistent with recommendations from several recent international meetings, including a shift towards herd-based measures, and clear differentiation between activities (and outcomes) relating to surveillance and to control. These measures will be incorporated within the national reporting system, to provide decision-makers with timely and objective information relevant to programme management, and further progress towards bovine tuberculosis control and eradication. The objectives of this study were to define performance measures to assist with ongoing programme review, create automated means to enable ongoing measurement, and to create an episode based summary file of bovine tuberculosis (TB) restrictions for the period 1989-2009. Progress has been made in correctly identifying, classifying and summarising periods of possible *Mycobacterium bovis* infection as indicated by TB test result data. Measures based on restriction based episodes have been defined and suggestions made as to how these can be summarised in a way that provides valid identification of trends and comparisons between regions.

Public health

An outbreak of tuberculosis affecting cattle and people on an Irish dairy farm, following the consumption of raw milk

Doran, P.¹, Carson, J.², Costello, E.³, More, S.J.⁴

¹ Department of Agriculture, Fisheries and Food, ² Wexford General Hospital,

³ Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food, ⁴ UCD CVERA

Irish Veterinary Journal 62, 390-397 (2009)

Bovine tuberculosis is an ongoing problem in Ireland, and herd incidence has remained at approximately 5% for some years. Spillover of infection from cattle to people remains an ever-present possibility, given the ongoing pool of infection in the Irish cattle population. This paper describes an outbreak of tuberculosis affecting cattle and people on a dairy farm in southeastern Ireland following the consumption of milk from a seven-year-old cow with tuberculous mastitis. Twenty-five of 28 calves born during autumn 2004 and spring 2005 were subsequently identified as TB reactors, and five of six family members were positive on the Mantoux test. During 2005, milk from this cow had mainly been used to feed calves, and was added only occasionally to the bulk tank. Therefore, the calves each received infected milk on an almost continuous basis between birth and weaning. The family collected milk from the bulk milk tank, and consumed it without pasteurisation. This case highlights the risks associated with the consumption of raw milk. In this family, TB has had a very significant impact on the health of two young children. These risks are well recognised, and relevant information for farmers is available. It is of concern, therefore, that raw milk consumption remains prevalent on Irish farms. New strategies are needed, in partnership with industry, to address this important issue.

Printed with permission from the Irish Veterinary Journal.

North-South linkages

An all-island approach to mapping bovine tuberculosis in Ireland

McGrath, G.¹, Abernethy, D.², Stringer, L.^{2, 3}, More, S.J.¹

¹ UCD CVERA, ² Department of Agriculture and Rural Development, Belfast, Northern Ireland,

³ Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand

Irish Veterinary Journal 62, 192-197 (2009)

This study used techniques in Geographical Information Systems (GIS) to explore the spatial patterns of bovine tuberculosis (TB) in the whole island of Ireland over an 11-year period. This is the first time that data pertaining to TB from the Republic of Ireland and Northern Ireland have been collated and examined in an all-Ireland context. The analyses were based on 198,156 point locations representing active farms with cattle in Northern Ireland and the Republic of Ireland between the years 1996 and 2006. The results consist of a series of maps giving a visual representation of cattle populations and associated detected bTB levels on the island of Ireland over this time interval.

Printed with permission from the Irish Veterinary Journal.

Bovine tuberculosis trends in the United Kingdom and Ireland, 1995 to 2008

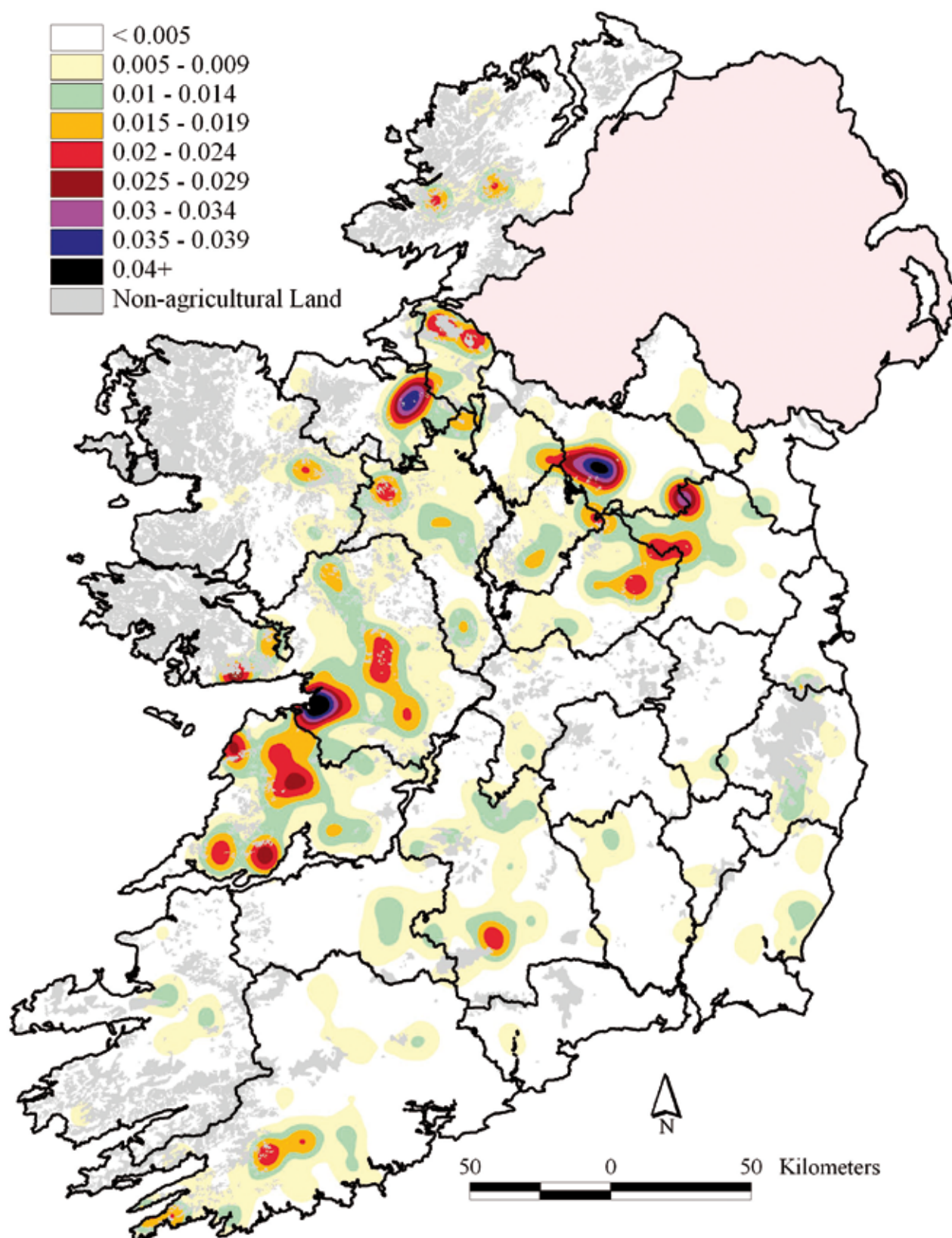
Downs, S.¹, Broughan, J.¹, Clifton-Hadley, R.¹, Goodchild, T.¹, Upton, P.¹, de la Rua-Domenech, R.², Rolfe, S.³, Blissett, M.⁴, Abernethy, D.⁵, Menzies, F.⁵, Clark, N.⁵, More, S.J.⁶, Higgins, I.⁶, McGrath, G.⁶, Duignan, A.^{6, 7}

¹ Veterinary Laboratories Agency, Weybridge, Surrey, England, ² Department of Environment, Food and Rural Affairs, United Kingdom,

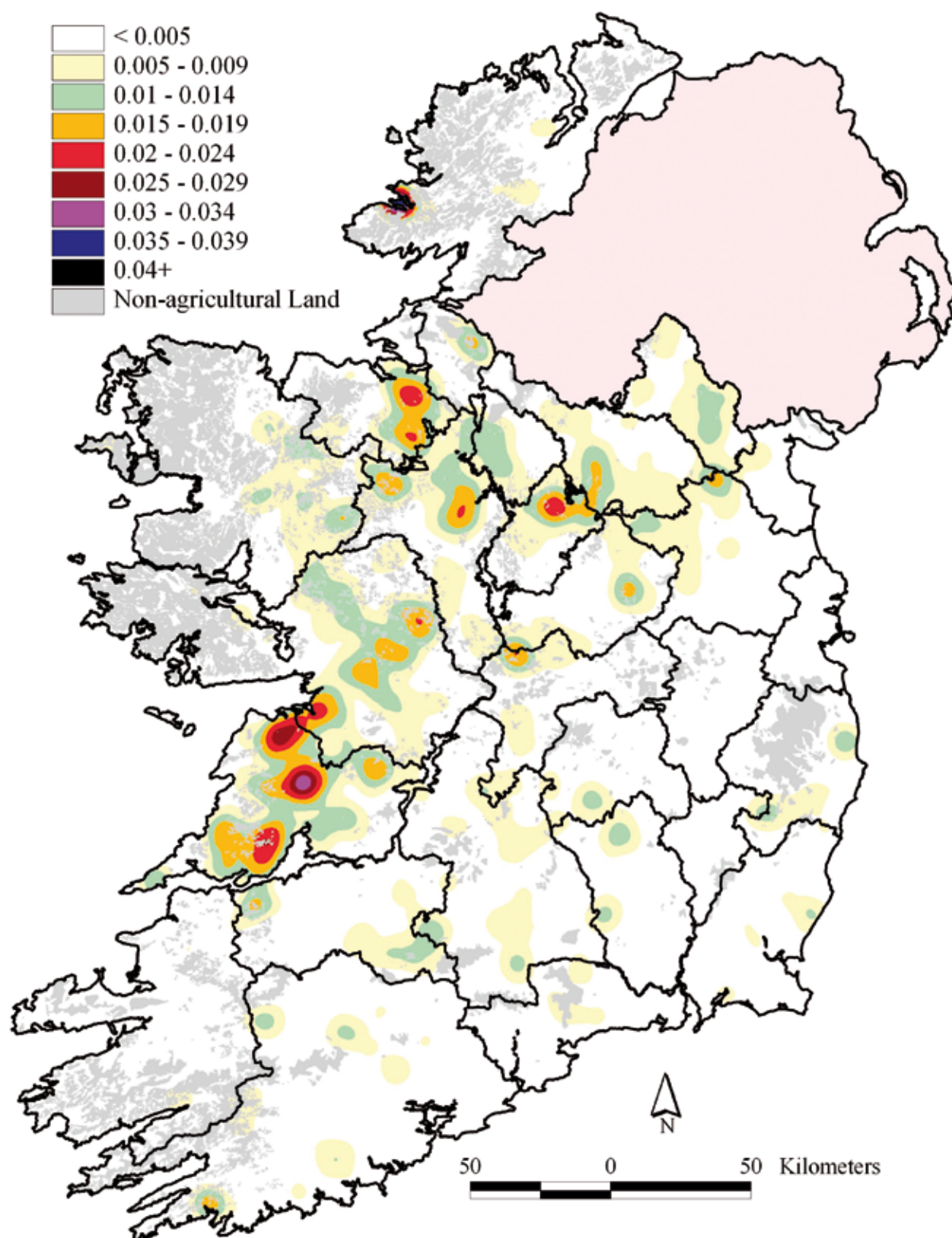
³ Welsh Assembly Government, Cardiff, Wales, ⁴ Scottish Government, Edinburgh, Scotland, ⁵ Department of Agriculture and Rural Development, Belfast, Northern Ireland, ⁶ UCD CVERA, ⁷ Department of Agriculture, Fisheries and Food

Different strategies in national bovine tuberculosis (BTB) control programmes are employed in Great Britain (namely England, Scotland and Wales), Northern Ireland and the Republic of Ireland, reflecting differences in perceived risk factors and programme priorities. Comparing the effects of these strategies or underlying BTB trends between the jurisdictions is hampered by different case definitions and test parameters used within each jurisdiction. This project is being conducted to address these concerns, with the aim to describe long-term BTB trends in the UK and Ireland during 1995 to 2008.

Density of TB incidence

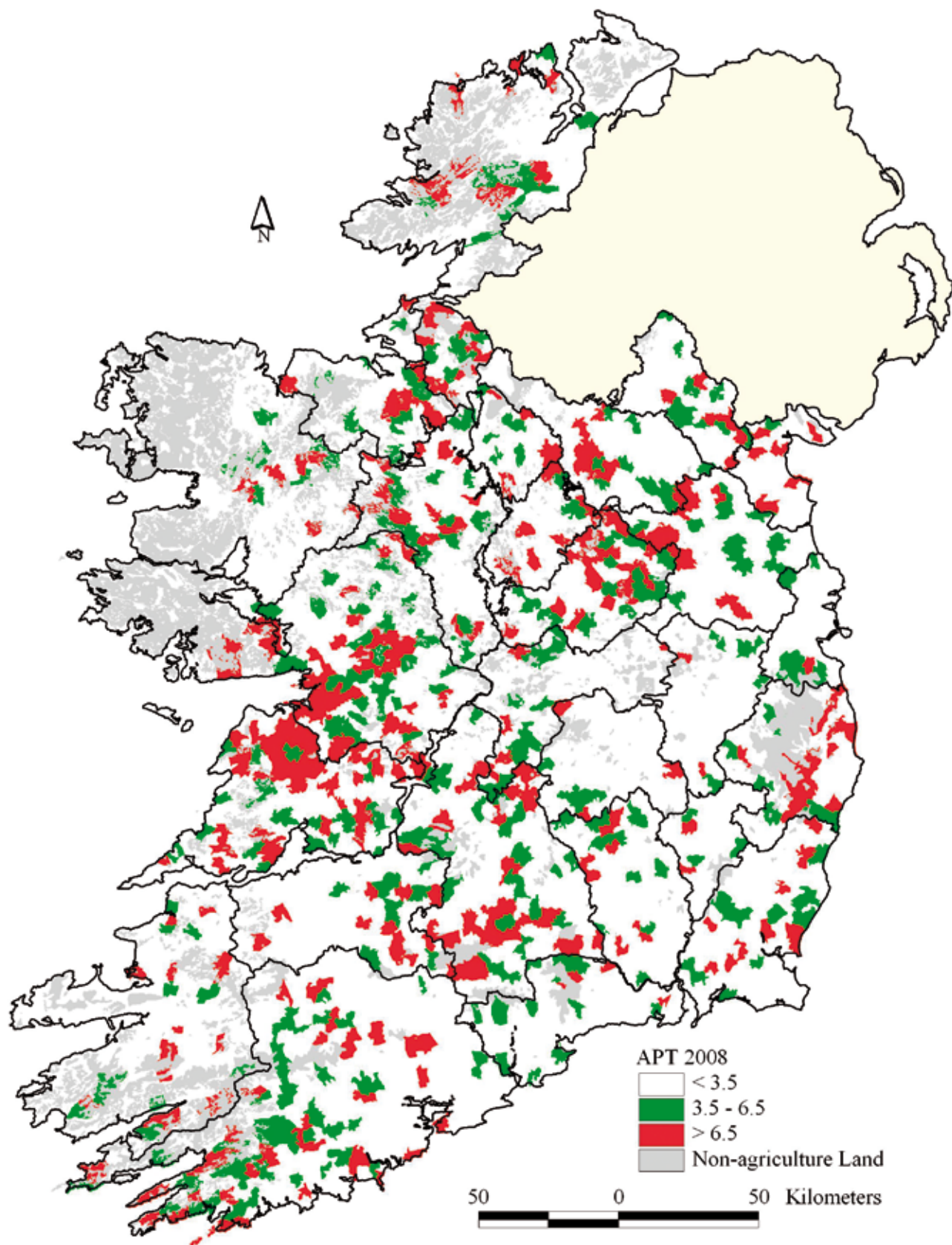


Density of TB incidence per square km during 2008 (kernel density with search radius at 10km).

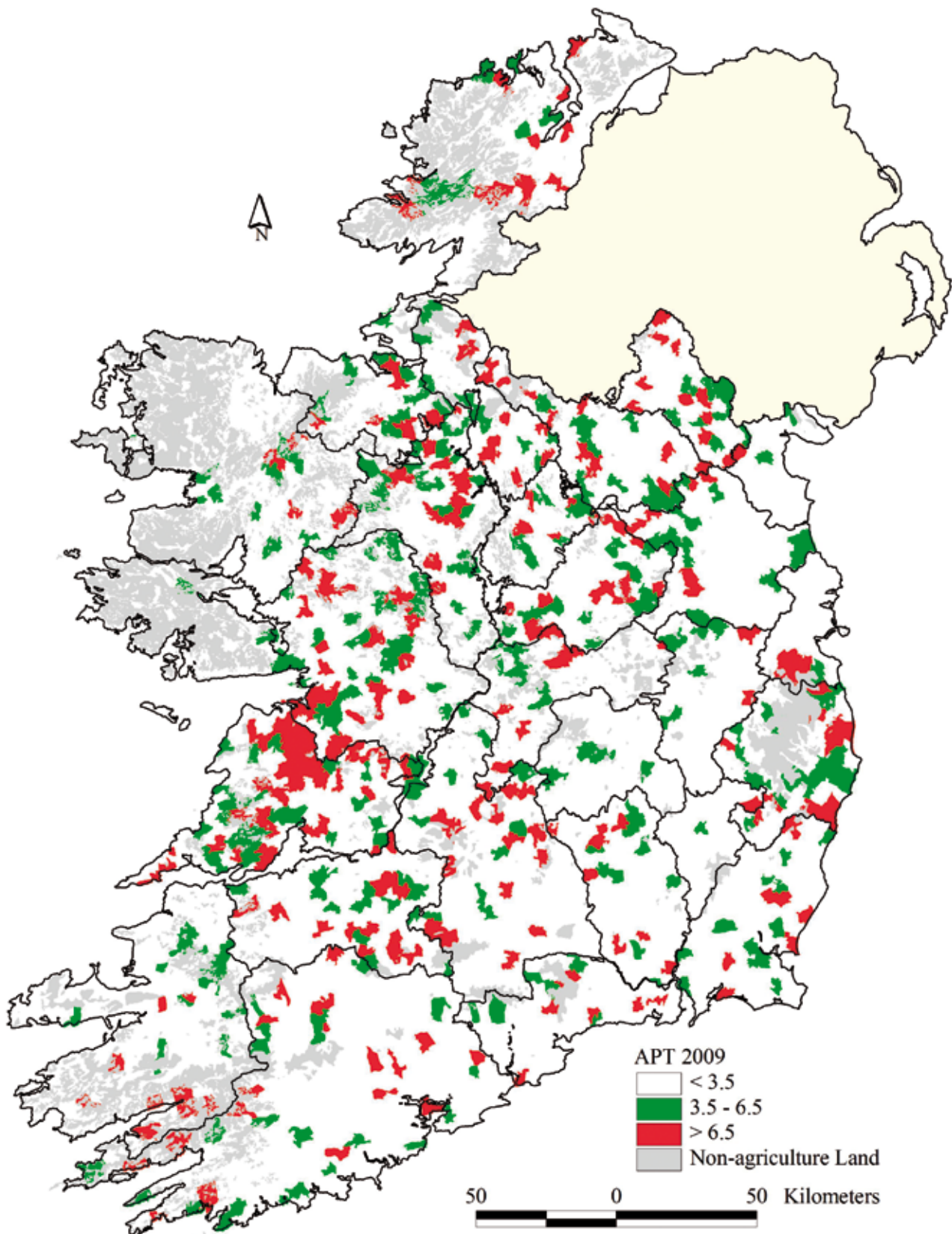


Density of TB incidence per square km during 2009 (kernel density with search radius at 10km).

APT per DED



APT (reactors per 1000 tests) per district electoral division, 2008.



APT (reactors per 1000 tests) per district electoral division, 2009.